# HOMOGENEITY TESTING

## An alternative approach

# ISO/IEC 17043:2010

**4.4.3 Homogeneity and stability**

**4.4.3.1** Criteria for suitable homogeneity and stability shall be established and shall be based on the effect that inhomogeneity and instability will have on the evaluation of the participants' performance.

**4.4.3.2** The procedures for the assessment of homogeneity and stability shall be documented and conducted, where applicable, in accordance with appropriate statistical designs. Where possible, the proficiency testing provider shall use a statistically random selection of a representative number of proficiency test items from the whole batch of test material in order to assess the homogeneity of the material.

**4.4.3.3** The assessment of homogeneity shall normally be performed after the proficiency test items have been packaged in the final form and before distribution to participants unless, for example, stability studies indicate that they should be stored in bulk form.

**4.4.3.6** In circumstances where homogeneity and stability testing is not feasible, the proficiency testing provider shall demonstrate that the procedures used to collect, produce, package and distribute the proficiency test items are sufficient for the purpose of the proficiency testing.

# ISO 13528:2005

**4.4 Homogeneity and stability of samples** (see ISO/IEC Guide 43-1:1997, 5.6.2 and 5.6.3)

Methods are given in Annex B for checking that the samples to be used in a proficiency test are adequately homogeneous and stable.

When a method of sample preparation is used such that the homogeneity criterion in Annex B is not met, then replicate samples shall be tested by the participants, or the standard deviation for proficiency testing shall include an allowance for the heterogeneity of the samples, as described in Annex B.

**Annex B**
(normative)

**Homogeneity and stability checks of samples**

**B.1 Procedure for a homogeneity check**

**B.2 Assessment criterion for a homogeneity check**

# QUESTIONS

1) **Should homogeneity testing be performed on each proficiency test item?**

2) **Should homogeneity be tested for each parameter?**

3) **Should homogeneity testing be performed by the EQA organizer themselves or can this be outsourced?**

4) **How to control the costs?**

# ECAT APPROACH

### 4.4.2 Preparation of proficiency test items

**4.4.2.1** The proficiency testing provider shall establish and implement procedures to ensure that proficiency test items are prepared in accordance with the plan described in 4.4.1.

NOTE It is advisable that the proficiency testing provider give due consideration to the preparation of sufficient numbers of proficiency test items, in order to allow for the need to replace any such proficiency test items lost or damaged during distribution, or intended to be provided for use after the results of the proficiency testing scheme have been evaluated. Such uses can include training aids for participants or use as a reference material.

**4.4.2.2** The proficiency testing provider shall establish and implement procedures to ensure appropriate acquisition, collection, preparation, handling, storage and, where required, disposal of all proficiency test items. The procedures shall ensure that materials used to manufacture proficiency test items are obtained in accordance with relevant regulatory and ethical requirements.

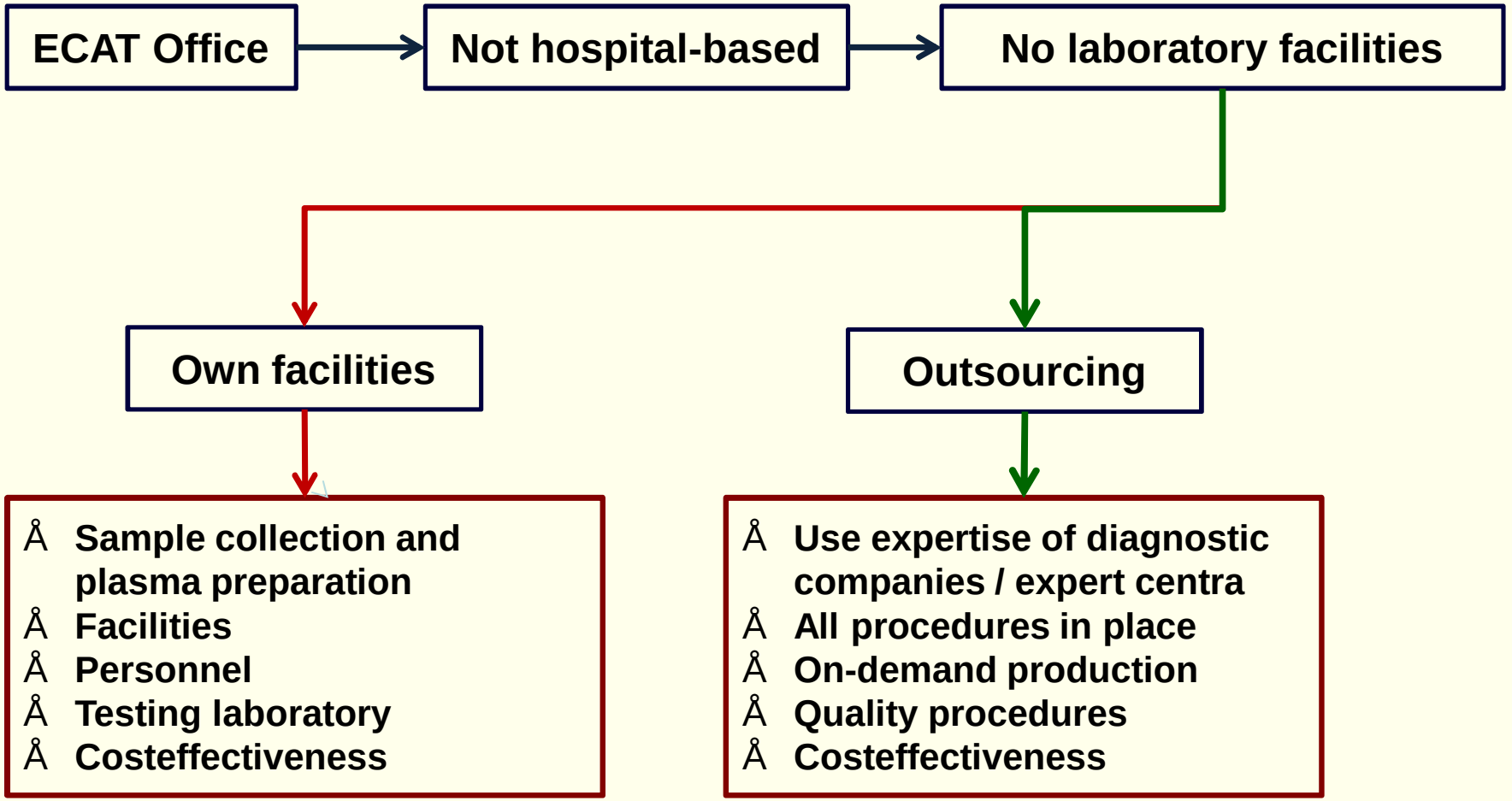
**4.4.2.3** Proficiency test items should match in terms of matrix, measurands and concentrations, as closely as practicable, the type of items or materials encountered in routine testing or calibration.

**4.4.2.4** In proficiency testing schemes that require participants to prepare or manipulate, or both prepare and manipulate, the proficiency test item and submit it to the proficiency testing provider, the proficiency testing provider shall issue instructions for preparation, packaging and transport of the proficiency test item.

### Subcontracting

**5.5.2** The proficiency testing provider shall not subcontract the planning of the proficiency test scheme (see 4.4.1.2), the evaluation of performance (see 4.7.2.1) or the authorization of the final report (see 4.8.1).

# ECAT APPROACH



# OUTSOURCING PRODUCTION

1) **Assessment of competence producer:**
   - source of samples
   - ethics
   - procedures for blood collection and handling
   - storage
   - production facilities and capacity
   - on-demand production
   - QC
   - Documentation / certificates

2) **Agreement**
   - includes the conditions for the production and delivery of samples.

3) **Annual plasma plan**
   - Description sample, amount, volume, delivery date, expiry date, key parameter(s), homogeneity criterion.

# OUTSOURCING PRODUCTION

## 4) Criteria for delivery and certificates

- no labels
- safety testing
- stability testing
- homogeneity testing

# EXAMPLE PLASMAPLAN

| Description | Conc/Act range | Unit | Key parameter | Number of vials | Delivery date | Volume (mL) | At least tenable until | Homogeneity (%) |
|---|---|---|---|---|---|---|---|---|
| Coagulation Control Normal | normal | | APTT, PT, ATIII, FVIII | 5000 | 01-09-2014 | 1.0 | 12-2015 | ≤ 3% |
| Coagulation Control AK | INR ~3 | | INR | 1500 | 15-01-2014 | 1.0 | 12-2015 | ≤ 3% |
| AK Calibrant (INR ~2) | INR ~2 | | INR | 275 | 15-01-2014 | 1.0 | 12-2015 | ≤ 3% |
| AK Calibrant (INR ~4) | INR ~4 | | INR | 275 | 15-01-2014 | 1.0 | 12-2015 | ≤ 3% |

# CRITERIA FOR DELIVERY AND CERTIFICATE

ECAT FOUNDATION

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## CRITERIA FOR THE DELIVERY AND CERTIFICATE OF ECAT PROFICIENCY TESTING ITEMS

Criteria for delivery of proficiency testing items:

- Test item should be identical to the ordered test item.
- Should be delivered at the indicated delivery date in the order.
- Vials should be without labels.
- The test items should be delivered at the following address:
  ECAT Foundation
  Dobbeweg 1
  2254 AG VOORSCHOTEN
  The Netherlands
- A certificate of analysis must be available.

*The ECAT Foundation should be informed timely when the proficiency test item/delivery doesn't fulfill all of the above mentioned conditions.*

# CRITERIA FOR DELIVERY AND CERTIFICATE

Criteria for certificate of analysis:

The certificate of analysis must include the following information:

- Appropriate name of the test item
- Type of material (plasma, serum, etc.)
- Lot number
- Volume
- Date of manufacturing
- Expiry date
- Information about safety testing
  - At least the following tests have to be performed: $HB_sAg$, HIV and HCV
  - Criterion: safety test have to be negative
- Information about homogeneity testing
  - The number of test items used for homogeneity testing. Criterion for minimum number of vials is given in table 1 (see overleaf).
  - Test parameter used for homogeneity testing, including result and unit.
  - Criterion for acceptance must be mentioned.
  - Conclusion

# HOMOGENEITY TESTING

## Å Criteria for homogeneity testing

Tabel1: Minimum number of vials tested per criterion

| | Batch volume (vials) | | | |
|---|---|---|---|---|
| **Criterion** | **>1000** | **200-1000** | **100-199** | **<100** |
| **Homogeneity** | 10 | 10 | 10 | 5 |
| **Stability (after reconstitution)** | 5 | 5 | 2 | 2 |
| **Long-term stability** | 5 | 5 | 2 | 2 |
| **Value assessment** | 10 | 10 | 5 | 3 |

## Å Excel application developed by the University of Stuttgart

# HOMOGENEITY TESTING

## Homogeneity check (ISO 13528 Annex B)

| Sample t | value#1, $x_{t,1}$ | value#2, $x_{t,2}$ | sample average (B.4), $x_{t.}$ | between-test-portion ranges (B.5), $w_t$ |
|---|---|---|---|---|
| 1 | 10.5 | 10.4 | 10.45 | 0.1 |
| 2 | 9.6 | 9.5 | 9.55 | 0.1 |
| 3 | 10.4 | 9.9 | 10.15 | 0.5 |
| 4 | 9.5 | 9.9 | 9.7 | 0.4 |
| 5 | 10 | 9.7 | 9.85 | 0.3 |
| 6 | 9.6 | 10.1 | 9.85 | 0.5 |
| 7 | 9.8 | 10.4 | 10.1 | 0.6 |
| 8 | 9.8 | 10.2 | 10 | 0.4 |
| 9 | 10.8 | 10.7 | 10.75 | 0.1 |
| 10 | 10.2 | 10 | 10.1 | 0.2 |
| 11 | 9.8 | 9.5 | 9.65 | 0.3 |
| 12 | 10.2 | 10 | 10.1 | 0.2 |
| 13 | | | | |
| 14 | | | | |
| 15 | | | | |
| 16 | | | | |
| 17 | | | | |
| 18 | | | | |
| 19 | | | | |
| 20 | | | | |

# HOMOGENEITY TESTING

| | |
|---|---|
| number of samples g | 12 |
| general average (B.6) $\bar{\bar{x}}$ | 10.02083333 |
| STD of sample averages (B.7), $s_x$ | 0.340092456 |
| within-samples STD (B.8), $s_w$ | 0.247487373 |
| between-samples STD (B.9), $s_s$ | 0.291612549 |

Expected standard deviation for proficiency assessment $\hat{\sigma}$ 1.1

Homogeneity ok

# CERTIFICATE

| Analytical data (Batch Size N = ≈420) | Specifications |
|---|---|
| **1. Within lot reproducibility**<br>1 ml n= **10** C1(OD) : **2,159** CV: **1,5 %**<br>n= **10** C1(ng/ml) : **89** | **For N ≥ 100 : n ≥ 10**<br>**For N < 100 : n ≥ 5**<br>CV (OD) ≤ 2%<br>☒ Passed in compliance |
| **2. Concentration and acceptance range**<br>Controls: C1 — n of vials: 14 — [C] ng/mL: 110 — SD: 8,4 — Acceptance range (ng/mL): 90-130 | **For N ≥ 200 : n ≥ 10**<br>**For 200 > N ≥ 100 : n ≥ 5**<br>**For N < 100 : n ≥ 3**<br>**C1**: ≈ 100 ng/mL [90-110 ]<br>☒ **Passed in compliance** |
| **3. Aspect and information about safety testing**<br>A ☒ B ☒ C ☒<br>* Each donor unit used for the preparation of control plasmas is a human plasma, which has been tested with registered methods for the presence of Hepatitis B Surface Antigen, Hepatitis C Antibodies (HVC) and antibodies to HIV 1 and 2 and was found negative. | **A**) Slightly opalescent to clear<br>**B**) No coagulum<br>**C**) Stable solution<br>Safety test have to be negative |
| **4. Stability of reconstituted reagents** Méthod : **STAR**<br>Fresh / 2 / 101 ng/mL<br>4h RT / 5 / 104 ng/mL | **n ≥ 2**<br>**4h at RT :**<br>110 ng/ml [90-130]<br>☒ **Passed in compliance** |
| **5. Stability of lyophilised plasma** Méthod : **STAR**<br>3 wks / 30°C / 5 / 120 ng/mL / 9% ΔC | **n ≥ 5**<br>**3 wks at 30°C:** Δ [C] ≤ 10%<br>☒ **Passed in compliance** |

| Analytische Verfahren<br>Analytical Procedure | | Anforderung<br>Requirement | Ergebnis<br>Result | |
|---|---|---|---|---|
| Reconstitution volume | | not applicable | 1 mL | |
| Type of material | | not applicable | plasma | |
| pH | | 7.0 – 7.8 | 7.6 | OK |
| Residual moister | | ≤1% | 0.73% | OK |
| $OD_{320nm}$ | | ≤20 | 10.9 | OK |
| Virology | HBsAg<br>HIV-Ab<br>HCV-Ab | Negative<br>Negative<br>Negative | Negative<br>Negative<br>Negative | OK |
| Homogeneity<br>Vials n=10 | Technoclot PT Plus (%) | CV ≤ 3% | 1.10% | OK |
| Batch specific value<br>Vials n=10 | Technoclot PT Plus (%)<br>CV=1.10% | 31.1 – 38.1% | 34.6% | OK |
| | Technoclot PT Plus (INR)<br>CV=1.10% | 2.22 – 2.72 | 2.47 | |
| Reconstituted stability based on Technoclot PT Plus (%)<br>4 hours at RT<br>Vials n=5 | | < 10% | 4.8% | OK |
| Storage stability based on Technoclot PT Plus (%)<br>1 week at 37°C<br>Vials n=5 | | < 10% | 0.8% | OK |

# HOMOGENEITY TESTING CONFIRMATION

- **Selection of samples is additionaly tested by a reference laboratory**
- **Reference laboratory is accredited according to CCKL / ISO 15189**
- **Contract / Instructions**
- **Excel report form**
- **Excel application developed by the University of Stuttgart**

# HOMOGENEITY DATA

| Sample | Parameter | Company | Ref. Lab |
|---|---|---|---|
| 220 | APTT | 1.3% | 0.4% |
| 221 | APTT | 1.7% | 0.3% |
| DD-64 | D-Dimer | 6.8% | 1.0% |
| H-60 | Anti-Xa | 1.6% | 4.7% |
| H-64 | Anti-Xa | 1.0% | 0.3% |

# HOMOGENEITY DATA

| Sample | Parameter | Ref. Lab |
|---|---|---|
| 220 | APTT | 0.4% |
| | Fbg | < 0.1% |
| | ATIII | 1.0% |
| 221 | APTT | 0.3% |
| | Fbg | 0.7% |
| | ATIII | 1.2% |
| 266 | APTT | 0.3% |
| | Fbg | 1.4% |
| | ATIII | 0.5% |

# RESPONSIBILITY ECAT

- **Confirmation of proper use procedures and correctness of certificates by producer of QC samples.**

- **Confirmation of proper use of procedures and correctness of reported results by reference laboratory**

**AUDITS**

# CONCLUSION

- **The alternative approach used by ECAT is a cost-effective manner to fulfil to the homogeneity requirements of the ISO 17043 standard.**
- **Establishment of homogeneity for every measurand is not necessary as long as you are able to demonstrate that the key parameter is representative for other measurands as well.**
- **So far the homogeneity is tested on each produced test item.**