Interlaboratory comparison and intra-laboratory checks

No proficiency-testing scheme covers the methods on a peptide, so each is compared with an independent accredited laboratory on a split lot, scored by E_n and zeta, published whatever the outcome and investigated when it fails; under it, the QC check material, the blind re-test of a retained vial and the two-analyst replicate.

Not yet approved
No approval of this revision is on file. This revision states what the laboratory's results system enforces today; it takes effect when a named reviewer approves it.

Document	
Number	SOP-11
Revision	1, first issue
Status	Not yet approved
Effective	Not yet in effect; takes effect when approved
Applies to	Every method; every comparison, re-test and replicate
Supersedes	Nothing; first issue
Fingerprint	a805 288c 65c2 1bc3

Laboratory	
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1 Purpose

To compare each of the five methods with an independent accredited laboratory on a split lot, score the agreement by E_n and zeta, publish the outcome whatever it is, investigate a disagreement as nonconforming work, and keep the daily layer under it: the QC check material, the blind re-test of a retained vial and the two-analyst replicate.

2 Scope

Every method the laboratory reports (SOP-02 to SOP-06) and every analyte of the scope table, grouped into five families by what changes the measurement (mass range, acylation, medium, chromophore). No proficiency-testing scheme covers any of the five methods on a synthetic peptide: the providers' own pages were read on the date ILC_PROGRAMME.md section 1 records, and the nearest rounds test the technique on another matrix. The comparison here is the "interlaboratory comparison other than proficiency testing" of ISO/IEC 17025:2017 7.7.2 b), a small comparison run by applying the relevant clauses of ISO/IEC 17043, with the written justification an accreditation body assesses kept in data.json quality.ilc.justification (not yet written). Where a technique-level round exists it is added as a second layer, never substituted; whether one is bought is an owner fact.

The families

Family	What makes them one	Analytes (data.json scope.rows)
F1	Lipidated incretin analogues	Semaglutide, Tirzepatide, Retatrutide
F2	GH-axis peptides	Ipamorelin, CJC-1295 (no DAC), Tesamorelin
F3	Melanocortins	Bremelanotide (PT-141), Melanotan II
F4	Short and fragment peptides	BPC-157, TB-500 (Tbeta-4 17–23), AOD-9604, Epitalon (AEDG), GHK (sold as GHK-Cu)
F5	Thymosin alpha-1	Thymosin alpha-1 (thymalfasin)

3 Responsibilities

Role	Responsibility
Reviewer	Qualifies the partner, chooses the lot and the re-test vials, types the partner's values from its report once and checks them against the report's hash, scores the comparison and signs the record; opens the CAPA on a failure.
Analyst	Runs the comparison item, the re-tests and the replicates as ordinary samples under the method's procedure, without sight of the partner's result.
Management	Names the partner, approves the budget and the calendar, decides whether an unnamed partner is acceptable and whether a failed comparison gates issue, and reads the results at the management review.

Named: Timothy Ian Benedicto, Medical Laboratory Technician; Roxanne Manlapaz, Laboratory Technician.

4 References and controlled sources

Standards and other documents

- ISO/IEC 17025:2017 7.7.1 and 7.7.2 (monitoring the validity of results; interlaboratory comparison other than proficiency testing), 7.10 (nonconforming work), 8.9 (management review).
- ISO 13528 (statistical methods for proficiency testing: E_n, zeta, the homogeneity and stability checks) and the Eurachem guide to proficiency testing, 3rd edition (2021), for the scores with the scheme's assigned value replaced by the other laboratory's; ISO/IEC 17043:2023 for the definitions; EA-4/21 INF:2026 (small interlaboratory comparisons); ILAC-P9:01/2024 (the justification when no scheme exists).
- ILC_PROGRAMME.md: the survey, the design and the worked figures this procedure follows; SOP-07 (the records), SOP-08 (the CAPA), QM-01 (the partner's impartiality), QM-00 7.7.

Controlled sources, read at every build

- data.json quality.ilc: the partner, its accreditation and independence statement, the criterion, the plan and the results, the re-test and replicate frequencies.
- lab/records/ilc.jsonl: the chained event log of every comparison, re-test and replicate, once it exists (it does not yet).
- lab/qc_trend.py (the QC check material series) and lab/management_review.py (the review that reads the results); lab/results.py (the uncertainties the scores are built from).

5 The partner laboratory

ISO/IEC 17025:2017 7.7.2, 6.6

A laboratory qualifies as the comparison partner for a method only when every line below holds, and the record of the qualification quotes the evidence for each. No partner is named yet (data.json quality.ilc.partner): the criteria are stated so that the owner's choice can be checked against them, and no laboratory is proposed here.

P1 Accredited	To ISO/IEC 17025:2017 by an accreditation body that is a signatory of the ILAC MRA, the certificate in force on the day the vials ship; kept: the certificate number, the body's online directory entry with the date read, the expiry, a copy of the certificate and scope.
P2 The test in scope	Read from the scope document, not the badge: for content and purity, assay or purity of an active substance, peptide or protein by HPLC-UV (or LC–MS for content); for water, Karl Fischer titration; for endotoxin, bacterial endotoxins by LAL; for identity, identification by mass spectrometry. A scope naming only other matrices does not qualify; kept: the scope line quoted with the document's address or hash.
P3 Reports U	The measurand compared, with its expanded uncertainty (k = 2) on the report, and how it was obtained on request; a partner that will not state U cannot be scored and is not a partner for that method.
P4 Independent	No common ownership, director, employee or premises; not a seller of peptides and not owned by one; not a supplier of the laboratory's reference standards or reagents for the method; no customer with a result pending; no fee contingent on the outcome. A signed statement from both laboratories, kept with QM-01's impartiality record and reviewed at the management review.

The partner laboratory, continued

P5 Agrees to publication	In writing, before the first shipment: its values, U and score published whatever the outcome; its report and its name published when it consents. Whether an unnamed partner is acceptable is an owner fact; this procedure recommends proceeding only with one that consents to be named.
P6 Can receive the item	In the same customs territory, unless the owner has checked the import rules for research peptides at the partner's end (data.json org.country is Canada).
P7 The same measurand	Purity as main-peak area percent at a stated wavelength and threshold; content as mass of free peptide per vial against a standard of stated content; water as a mass fraction; endotoxin as EU per mg of powder. A different technique for the same measurand is welcome; a different measurand is recorded and not scored.

What does not qualify: a badge on a web page; accreditation for another discipline; another seller's in-house laboratory; a laboratory that sells peptides; one that compares only if the result stays private.

6 The comparison item

6.1 The lot: one lot of one analyte, bought by the laboratory from a seller as a research chemical, never a customer's sample unless the sender asks in writing and accepts the publication rule. The seller is not told, is not named in the publication, and no seller with a pending accession supplies it. The lot may become the QC check material of a later series only after the comparison is published.

6.2 Vials and allocation: at least 20 sealed vials of one fill, each given a random position (the permutation and its seed recorded in the log) and allocated in that order: 5 to the laboratory's own analysis (5 vials (mass per vial 3; water content 2; HPLC from the mass per vial preparation; LC–MS from the HPLC preparation; endotoxin from the mass per vial preparation)), the same to the partner, 4 to the stability re-test, 2 to the partner as spares, the rest retained unopened.

6.3 Homogeneity: the lot was not filled for a comparison, so its vial-to-vial variation is measured, not assumed. Content: the per-vial gross fills and contents of every vial opened give the between-vial standard deviation s_s, compared with 0.3 times the combined standard uncertainty of the laboratory's own result; below it negligible, above it added to both laboratories' u in quadrature before the score, above u_c itself "not scored: inhomogeneous lot". Water: the four per-vial values, the same rule. Endotoxin: the per-vial values in ln space. Purity is a property of the bulk and is not tested per vial.

6.4 Stability: the four stability vials are measured on the day the partner's report is received (content on two, water on two) and compared with their t0 values; a difference within 0.3 u_c is negligible, a larger one is published with the comparison, and for water it means the score is read with that caveat and a second water comparison is scheduled on a fresh lot. A partner result more than 60 days after shipment is scored with the stability figure printed beside it.

6.5 Blinding, which the register proves: the partner receives vials labelled with the comparison code, the analyte, the nominal fill, the storage condition and the measurands to report, and is told neither the seller, the label claim nor any result. The laboratory logs the lot as an accession of its own, runs it in an ordinary sequence, reviews, issues and anchors it before the partner's report is opened; the log records the anchor and the time the partner's file was first read, in that order. The model does not yet carry a set kind for a comparison item (ILC_PROGRAMME M1); until it does the accession is an ordinary one and the certificate's notes say what it is.

6.6 Shipping: sealed vials in their original secondary packaging with a chain-of-custody sheet, by a tracked courier within 5 business days of allocation; Semaglutide, tirzepatide and retatrutide and any lot labelled 2–8 °C ship with a cold pack and a single-use temperature indicator; the partner confirms receipt date, condition and storage, and nothing the terms exclude is shipped.

7 What is compared, per method

Method	Measurand both report	Score	Recorded, unscored
Identity by LC–MS	Whether the item is the named analyte: the observed m/z of the main charge state, the mass error in ppm, the conclusion against the same claimed analyte	Agreement of the conclusion; one confirms and one does not: unsatisfactory, identity on hold	Each laboratory's mass error and charge state
Purity by RP-HPLC–UV	Main-peak area percent at the stated wavelength, peaks integrated above a stated threshold (ours 0.05% area)	E_n, both U; a difference is first a method difference to explain	Largest single impurity, count of peaks at or above 0.1%
Mass per vial	Mass of free peptide per vial against a standard of stated content and u_RS, mean of n vials (ours 3)	E_n, both U, with s_s when the homogeneity rule says so; the comparison this programme is most for, since the two standards are independent	The standard each used; the basis (free peptide on both, or not scored)
Water content	Water as mass percent of the powder as received (mean of 2 vials) and per vial	E_n, both U, read with the stability figure	Technique, oven temperature or solvent, blank, standard
Bacterial endotoxin	EU per mg of powder and EU per vial with the ×/÷ factor, or "below" the reporting limit	E_n in ln space (x to ln x, U to ln F); both below: an agreement of conformity, satisfactory but uninformative; one quantified and one below: satisfactory when the value is below the other's limit	Technique, lambda, dilution, lysate and CSE lots, PPC recovery

The endotoxin item when the lot is clean: most lots read below quantitation at both laboratories, which compares nothing. Either the split lot stays the item and the comparison is qualitative in most years, or two vials are reconstituted in LAL reagent water, spiked with the laboratory's CSE lot to a level inside both curves, dispensed into pyrogen-free tubes, frozen and shipped with a retained pair, both laboratories reporting EU/mL; the choice is an owner fact (data.json quality.ilc.plan, endotoxin_item).

8 The calendar

8.1 Two lots a year, each through all five methods with the one partner (or two, if no single scope covers the five): lot A is always family F1 and lot B rotates F4, F2, F3, F5, F4, so that every family is compared within three years; within a family the analyte rotates. The minimum the owner may fall back to is one lot per method per year; then lot A alone, the rotation stretches to six years, and the status page says so.

8.2 Timing: the first comparison after the instrument runs and the analyte's validation report is signed, never before, because an E_n needs the U the validation gives; thereafter lot A in the first half of each year and lot B in the second, so the management review reads one of each. The plan: none is planned yet (data.json quality.ilc.plan); the first follows the instrument running and the first validation report signed, and its date is an owner fact.

8.3 An extra comparison within three months after: an unsatisfactory score; a new instrument or column chemistry for purity or content; a new reference standard lot whose assigned content differs from the previous by more than its u_RS; a new analyst authorised before any two-analyst replicate; a change of the oven temperature or the lysate type; a complaint upheld on a measurement.

9 Scoring

For every quantitative measurand, with both laboratories' expanded uncertainties at k = 2 (a partner's other k converted to u = U/k and recorded; a result whose certificate prints nu_eff below 10 with a t factor converted to u = U/t, and the record says so); endotoxin in ln space; s_s added to both u in quadrature when the homogeneity rule applies:

$E_n = (x_{ours} - x_{theirs}) / \mathrm{sqrt}(U_{ours}^2 + U_{theirs}^2)$

zeta = $(x_{ours} - x_{theirs}) / \mathrm{sqrt}(u_{ours}^2 + u_{theirs}^2)$, u = U / 2 (for two laboratories zeta = 2 E_n)

Score	Satisfactory	Warning	Unsatisfactory
E_n	|E_n| at most 1.0	none	|E_n| above 1.0: unsatisfactory, a nonconformity and a CAPA
zeta	|zeta| at most 2.0	2.0 to 3.0: recorded, reviewed at the management review; two in a row on one method open a CAPA	|zeta| at or above 3.0

- The verdict is E_n's; zeta is printed beside it because the two scores carry the same information with different bands, and the zeta bands give the warning zone E_n lacks (ISO 13528 as the Eurachem guide reproduces it).
- The U sanity line: E_n rewards a wide U. Each measurand has a fitness target, the U a customer can use (ILC_PROGRAMME 2.5, provisional until the validated figures replace them); a satisfactory E_n reached with either laboratory's U above its target is printed "satisfactory, uninformative" and counts for clause 7.7.2 only once repeated with a U inside the target. An unsatisfactory E_n is never softened by this line.
- Unsatisfactory, in one list: |E_n| above 1 on any scored measurand; an identity disagreement; a content comparison the partner reports on another basis (not scored; repeated with the basis agreed, and the planning failure recorded); a lot found inhomogeneous or unstable beyond u_c (not scored; repeated on a new lot); a partner report without U (not scored; the partner fails P3); a publication later than 15 business days, a nonconformity of the programme that goes to the corrective-action log too.
- No function in the code computes the scores yet (ILC_PROGRAMME M2): until the model carries en_zeta the reviewer computes E_n and zeta by hand from the formulas above, a second person recomputes them, and both computations are in the record; the worked figures of ILC_PROGRAMME 2.5 are the check values.

10 When a comparison fails

ISO/IEC 17025:2017 7.10, 8.7

An unsatisfactory score is nonconforming work and opens a corrective-action case (SOP-08) on the day it is scored, with the comparison id as its source. The investigation, recorded step by step in the case:

10.1 Transcription and arithmetic on both sides: the value and U on each report against each result set; E_n recomputed by hand; the units and the basis.

10.2 The laboratory's own evidence first: the run's suitability and QC check material points; the standard's lot, assigned content and u_RS (content is the likeliest place); the dilution scheme as executed; the integration for purity; the oven temperature, blank and drift for water; the curve, PPC and dilution for endotoxin.

10.3 The retained vials: two re-tested blind by the other analyst (or the same analyst on another day), compared with the original by the re-test rule below; a re-test that disagrees with the original locates the problem in the run, one that agrees locates it in the method or the standard.

10.4 The partner's evidence: its method summary, standard, n, U budget, raw data on request; a method difference for purity is resolved by running the item on the other conditions where the column and gradient allow, and recorded as a method finding that may change SOP-02.

10.5 A third value where the steps above do not explain the gap: a second accredited laboratory or, for content, a standard of independent assignment; all three values published with the comparison.

When a comparison fails, continued

10.6 Impact on issued reports: every report issued with the method since the last satisfactory comparison or QC check is listed; where the bias found would change a conformity statement or move a result by more than its U, each sender is told and the report corrected by a new accession (SOP-08), and the corrections log carries it.

10.7 Root cause, action and effectiveness: the case closes only on a repeat comparison on a new lot within three months, scored satisfactory with a U inside the target, plus the QC series in control for 20 runs; never on the repair alone.

10.8 While it is open: identity on hold for that analyte; whether content results for the method are held or issued with the open comparison noted on the certificate and the site is an owner fact (data.json quality.ilc.gate); purity, water and endotoxin are issued with the open comparison noted; the hold is a status line from the day it starts.

10.9 Publication of the failure is not delayed by the investigation: the score goes up within the 15 business days as “under investigation, CAPA n”, and the resolution is added when the case closes.

11 Intra-laboratory checks

ISO/IEC 17025:2017 7.7.1

The comparison is one or two points a year; what makes them believable is the daily layer. Three controls, each a series, and what each catches: the QC check material a run that has drifted; the re-test a result that would not repeat weeks apart; the two-analyst replicate a person-dependent step; the comparison what none of the three can, a bias shared by everything in the building, which for content is the standard.

11.1 QC check material: a characterised in-house lot run with every sequence and measured as a sample (the reference-standard lot, or a previous comparison’s lot once published, for purity and content; a retained homogeneous powder lot for water; a lot of known endotoxin for LAL), its assigned value from at least ten runs and its limits frozen by the reviewer. lab/qc_trend.py charts it as its own series per method from each run’s qc_check record, judged against the lot’s limits in lab/qc_materials.json (QM-00 7.7); no lot is recorded yet.

11.2 Retained-vial re-test: once a month per method, one retained vial of an issued accession, chosen by the reviewer at random from those inside the retention period, is re-logged under a blind re-test code and run as a sample by the analyst; the result is compared with the original as $\text{zeta_r} = (x_{retest} - x_{original}) / \sqrt{u_{retest}^2 + u_{original}^2}$, warning beyond 2 and action beyond 3; for water, which an opened vial has changed, the re-test uses an unopened retained vial. A series whose zeta_r drifts one way is a bias in time, which is the point of charting it.

11.3 Two-analyst replicate: once a quarter per method, once a second analyst holds the authorisation, both analysts prepare and run the QC check lot independently on the same day and the pair is compared the same way; the series begins with the second authorisation.

11.4 Each re-test and replicate is a result set of its own, so the original’s audit trail is untouched and the pair is two hashed records. lab/qc_trend.py charts the pairs as their own series.

12 Publication

– Within 15 business days of both results being on hand, on the reports page beside the reports it compares, under its own anchor; the same rows as a file beside it and one row on the status page; a sentence on the methods page pointing at it; nothing on the home page until the first result exists. build.py does not yet render the section (ILC_PROGRAMME W1): until it does, the result is recorded in data.json quality.ilc.results and the log, and nothing is published by hand.

Publication, continued

- What an entry prints: the comparison id and the date of the partner's report; the analyte, the family, the lot described as a commercial lot bought for the comparison with the seller unnamed, the vial count and allocation; the partner by name with its accreditation body, certificate and the scope line quoted when it consents, else the body and scope line with the sentence that it asked not to be named and that an unnamed partner is worth less to the reader; per measurand both values with their U, s_s where applied, E_n, zeta and the verdict in words; for identity the two conclusions; both reports as PDF where the partner consents; the stability and homogeneity figures; for a failure the case, then the cause, the action, the repeat's score and any report corrected because of it; and per method "last satisfactory comparison" or "none yet".
- Rules: the partner's values are typed from its report once, checked by the reviewer against the report's hash, and the log holds the hash; nothing is removed, averaged, ranked or summarised into a badge. Until the first comparison the section prints the plan and "none completed".

13 Deviations and open points

- A departure from this procedure, planned or found afterwards, is nonconforming work and is handled under SOP-08. One that bears on a result is stated on the certificate, in the open point or the notes of the section concerned, never left out.
- Owner facts not yet supplied: the partner laboratory (none is proposed here), the budget, the first comparison's date, whether an unnamed partner is acceptable, the endotoxin item design, and whether a failed comparison gates issue (data.json quality.ilc).
- No comparison, re-test or replicate has been run; the log lab/records/ilc.jsonl does not exist until the first event is written.
- Not yet in the code, each said above where it applies, so this procedure's hand steps stand until they land: the model carries no set kind for a comparison item, a re-test or a replicate (ILC_PROGRAMME M1); no scoring function (ILC_PROGRAMME M2); the site does not render the comparisons (W1).
- The ISO 13528 clause numbers for the bands are cited from the Eurachem guide until the laboratory's own copy of the 2022 edition is on file.

14 Records

- data.json quality.ilc: justification, partner, accreditation, independence statement, publication agreement, criterion, plan and results.
- lab/records/ilc.jsonl: one chained line per event (planned, partner qualified, lot received, allocated, shipped, received by the partner, ours sealed, theirs received, stability tested, scored, published, case opened and closed, not scored, withdrawn), with the permutation seed, the shipment, the anchor of the laboratory's own result, the hash and first-opened time of the partner's report, the homogeneity and stability figures and the scores with their inputs; walked by lab/integrity.py with the other record logs and archived with them (SOP-07).
- The result sets of the comparison item, the re-tests and the replicates, with their certificates; the partner's reports and method summaries; the corrective-action cases.

15 Revision history

Revision	Change	Approval
1	First issue.	Not yet approved

The fingerprint in the control block is the SHA-256 of this document's text as composed, first 16 hex digits. A change in any controlled source changes it; a copy whose fingerprint differs from the approved one is not the approved text, and a change is approved as a new revision.

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Current revisions

End of document