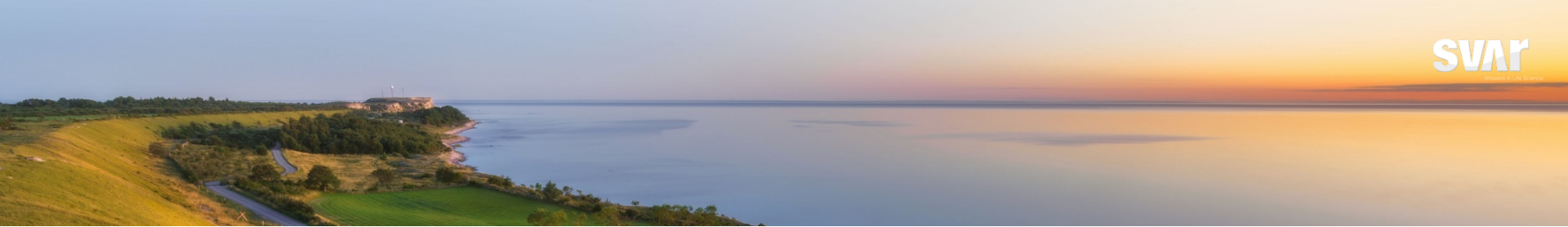SVAR LIFE SCIENCE

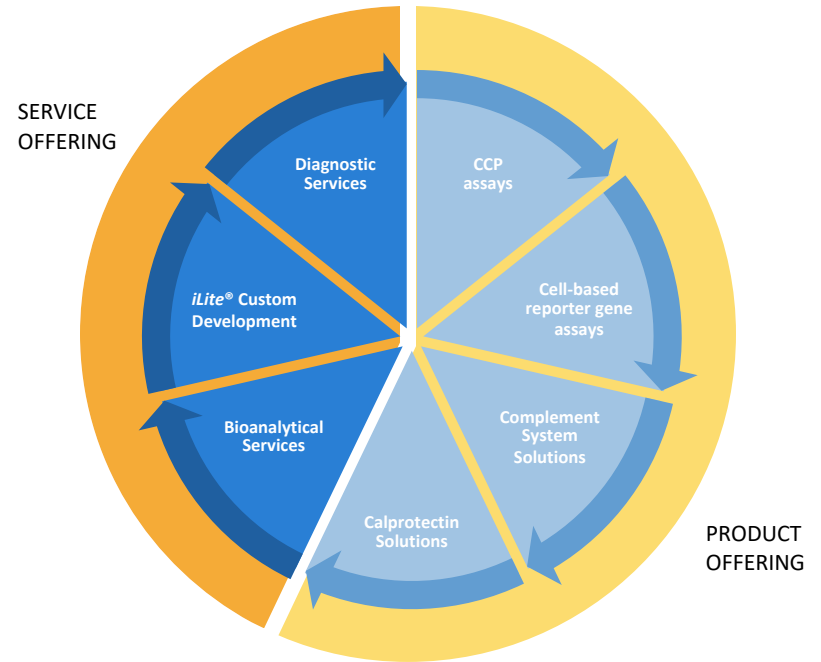# CLIA/ISO or BMV for Biomarkers and Diagnostics: two worlds?

SVAR LIFE SCIENCE

Svar offers a comprehensive product portfolio supporting the development of new drugs and providing tools for patient diagnosis and monitoring.

SVAR was formerly Euro Diagnostica, including Wieslab, Biomonitor and Calpro

Portfolio include diagnostic kits, research tools, diagnostic laboratory services and bioanalytical services.

SERVICE OFFERING

PRODUCT OFFERING

Diagnostic Services

CCP assays

Cell-based reporter gene assays

Complement System Solutions

Calprotectin Solutions

Bioanalytical Services

iLite® Custom Development

Topics

- Diagnostic laboratories ISO 15189 vs CLIA
- Diagnostic kits - IVD, CE and RUO
- Verification and validation for diagnostic method
- Laboratory quality control in diagnostic laboratories
- Diagnostic case for assay verification
- Considerations for drug development and biomarkers

Bioanalytical method validation is not in scope

- Guidelines for Bioanalytical method validation (BMV) should not be used for Biomarker assays
- Biomarker Assays ≠ Assay for Pharmacokinetics
- No guidelines exists for Pharmaceutical development and Biomarkers
- Why can we not use the easy 4-6-xx paradigm?
  - Biotherapeutics are recombinant proteins produced with high quality control and stability
  - A biomarker is an in vivo endogenous protein
  - Stability of biomarkers may vary
  - A recombinant variant of the biomarker do not necessarily fully reflect the in vivo endogenous protein
  - The validation should be performed for the Context of Use of the Biomarker

Ref. Joanne Goodman, Kyra Cowan - On behalf of the EBF at the EBF Open Symposium 2020

Medical diagnostic laboratories ISO 15189 vs CLIA

ISO 15189	CLIA
International Organization for Standardization (ISO) and required for diagnostic medical laboratories in international and in EU	Based on federal law in US. CLIA is required for US derived specimen AND only when the result drives a treatment decision for which the result is reported to physician
Voluntary in US	Labs outside US can register for CLIA
International expert consensus	Experts from FDA, CMS and CDC are involved
Focus on process based quality system: ✓ ISO inspectors perform periodic inspection on lab and quality management system ✓ 1-3 year cycles of external inspections ✓ Internal audits (self inspections) every year	Focus on procedure: ✓ Peer inspectors ✓ Self-inspections every year ✓ 2-year accreditation cycle

ISO 15189 can be substituted by ISO 17025 in some countries – ISO17025 is a general guide for analytical labs and ISO 15189 focus on medical laboratories

With inspiration from Schneider et. al. Ann. Lab. Med. 2017

Diagnostic labs, Biomarkers and development of drugs

- Diagnostic medical laboratories are frequently used for safety testing using standard clinical tests in clinical studies for approval of new drug candidates
- ISO 15189 and ISO 17025 lab data is acceptable for NDA at FDA
  - In a recent EBF survey 3 out 12 companies shared that they have used ISO 15189 or ISO 17025 laboratory data for registration of drugs
  - Most common markers where used for Patient safety and Subject inclusion/exclusion (eg tests for suspected immune-mediated allergic reactions, haematology biomarkers, pregnancy and HIV testing)
- CLIA is required when treatment decisions are needed for US patients

Diagnostic laboratories: what about validation and verification?

- The laboratory shall use only validated procedures to ensure that they are suitable for intended use
  - The laboratories often use use CE/IVD labelled kits but must document a <u>Verification</u> that the kit lives up to the manufacturers validation
- The validation and verification shall be as extensive as are necessary to meet the Intended Use in the given application
- Intended Use = Context of Use
- Method procedures need to be revalidated if conditions, intended use and technical advances are changed
- A validation/verification ensures an accredited status of test

ISO 15189:2012

When do a diagnostic laboratory make a full validation of diagnostic methods?

- Based on <u>Intended use</u> for the diagnostic purpose
- IVD/CE labelled: kit a verification is acceptable
- From ISO 15189: The laboratory shall validate procedures derived from the following sources:
  - ➤ non-standard methods
  - ➤ laboratory designed or developed methods
  - ➤ standard methods used outside their intended scope
 - ➤ validated methods that have been modified

ISO 15189:2012

IVD products and CE label

- The CE/IVD labelling of diagnostic kits is based on
  - ➢ EU Directives
  - ➢ 510(k) clearance for new In Vitro Diagnostic (IVD) tests
  - ➢ Other international regulations
- IVD/CE requires a full validation by manufacturer for the Intended Use of the product
- CE label is required in EU
- IVD symbol is used for non-EU countries
- Some products are sold as Research Use Only (RUO) which require no registration
- Research Use Only kits often have less published information for its Intended Use in Diagnostics

Typical parameters in a diagnostic validation for a quantitative method

- Linearity, Range of Measurement, Limit of detection/Limit of quantification
- Precision
- Accuracy: expressed as a Trueness and Measurement uncertainty
- Analytical Sensitivity and Specificity
  - ➤ Comparison between labs with well known specimens
- Clinical Sensitivity and Specificity
  - ➤ Normal range and disease range – a statistical relevant number of healthy vs disease samples
  - ➤ Typically at least 120 samples from healthy individuals
  - ➤ Number of disease individuals depends on disease (eg rare disease vs common disease)

- The methods are monitored by internal quality controls (IQC) and external quality assessment (EQA) or proficiency testing (PT)

Linearity and LOQ

- Linearity is most often performed on patient samples which equals Parallelism
- The Limit of quantification (LOQ) is the lowest level of analyte that can be determined with "Acceptable performance":
    - Often defined as: 3 × Standard Deviation
    - Acceptance should be defined *a priori* for its Intended Use
    - Can include precision, precision and trueness, or measurement uncertainty

- The samples used for establishment of LOD/LOQ should be either
    a) blank samples, i.e. matrices containing no detectable analyte
    b) test samples with concentrations of analyte close to or below the expected LOD/LOQ
- 10 replicates tests are often used

Accuracy/Trueness and Measurement uncertainty

- Accuracy/Trueness is the "closeness of agreement with a reference value"
- No fixed acceptance criteria - depends on the Intended use for the diagnostic purpose and determined *a priori* for each application
- Use appropriate reference materials
  - Examples include: positive, negative and normal controls, certified reference materials, EQA materials, synthetic samples or material characterized by another technique
  - Rarely use spiked samples!

Assay control performance: Internal tests by IQC

- At least 2 levels of IQC are used for quantitative testing
  - Depending on Intended Use
  - Eg one low and one high level in the linear working range
- IQC are used during validation to determine Precision and control ranges
- A full validation can include:
  - Acceptance performance determined *a priori*
  - Established in 6 Precision runs with multiple replicates
  - Calculate mean and SD and establish preliminary acceptance ranges (mean +/-2SD; mean +/- 3SD)
  - Additional investigation is performed in at least 20 assay runs over an extended time period to fix/adjust acceptance ranges
- A verification can be smaller depending on available data from kit producer and the intended use

Example on *a priori* determined acceptable performance for CLIA method validation:

Hematology CLIA 2019		
Analyte or Test	NEW Criteria for AP	OLD AP
Cell identification	80% or greater consensus	90% or greater consensus
White blood cell differential	TV ± 3 SD	Same
Erythrocyte count	TV ± 4%	TV ± 6%
Hematocrit	TV ± 4%	TV ± 6%
Hemoglobin	TV ± 4%	TV ± 7%
Leukocyte count	TV ± 5%	TV ± 15%
Platelet count	TV ± 25%	Same

www.westgard.com

Continuous assay performance indicator: Westgard and Levy-Jennings

- Follow IQC performance by Control charts – Levy-Jennings
- Example of Westgard rules
  - Within 2 SD: run is accepted
  - Outside 2 SD and within 3 SD. One time observed as a random error. At repeated times in consecutive runs: Corrective action
  - Outside 3 SD : Corrective action
  - Assay drift: When IQC gives repeated results on one side of the mean in 10 consecutive runs: Corrective action

www.westgard.com
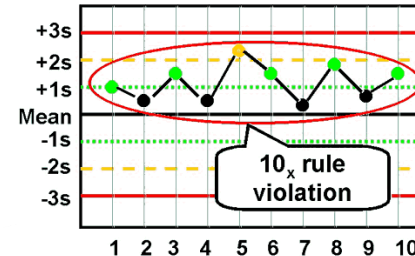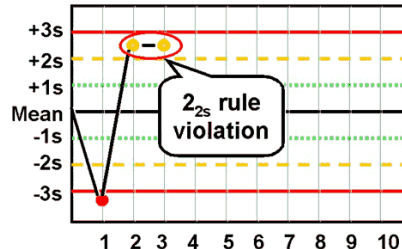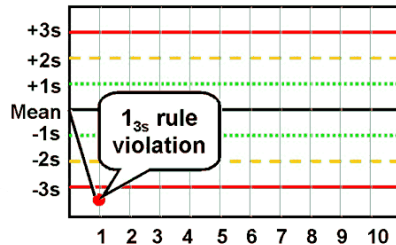
Real data example
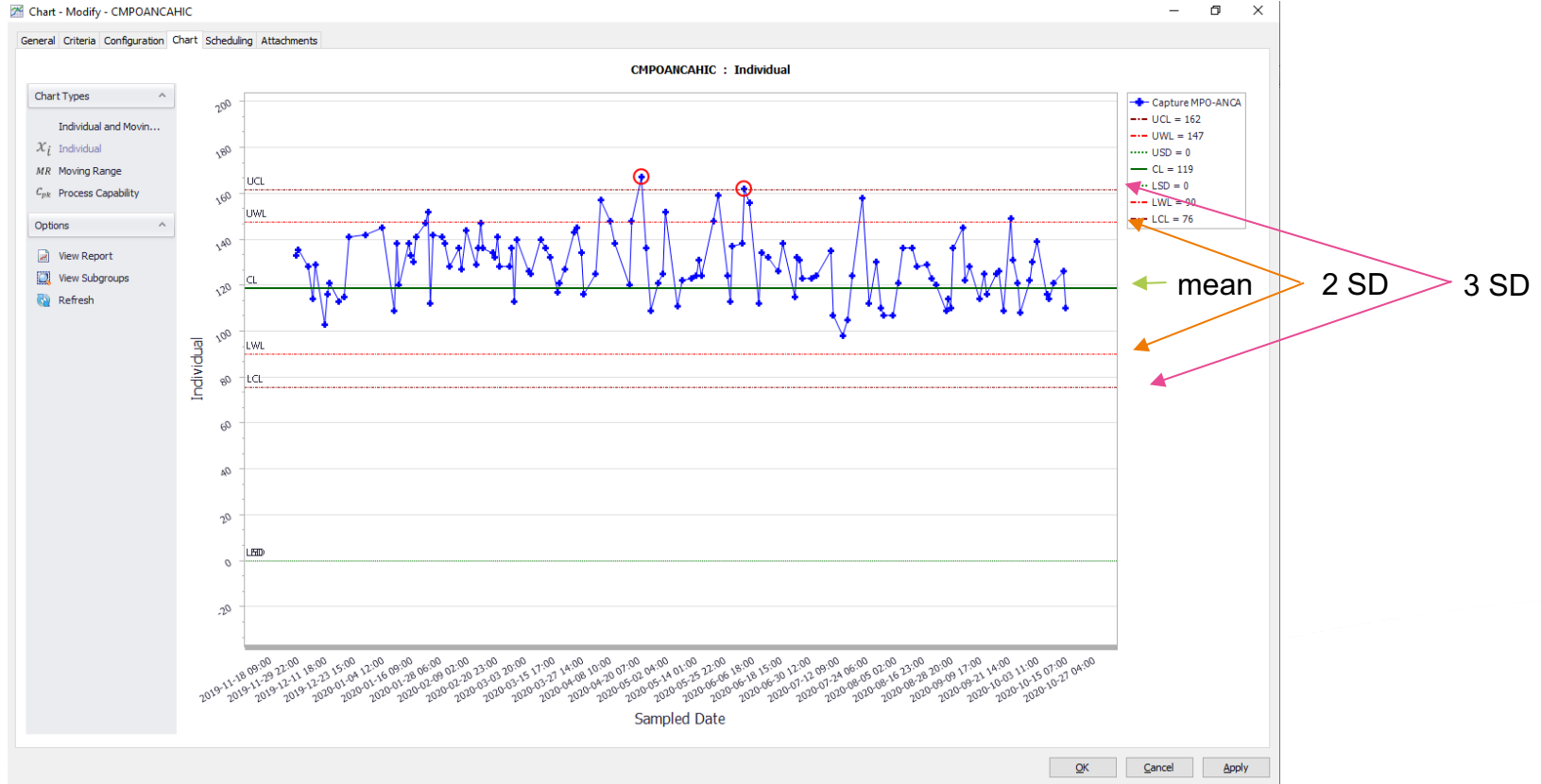
External quality assessment (EQA) and proficiency testing (PT)

- Programs for external quality assurance monitored by authorities
  - CLIA: CMS
  - ISO: eg Instand in Germany, UKNEQAS in UK
- For analytes were no EQA/PT exists it is also acceptable to run inter lab testing at regular intervals
- Samples are usually true patient samples and should include pre-analytical sample collection
- Performance is controlled at periodic authority inspection
- Deviating results shall result in corrective actions and labs can loose their accreditation

EQA are important to determine accuracy of diagnostic tests
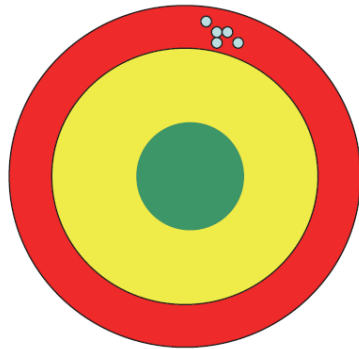
Precise, but Inaccurate
IQC OK
EQA rejected
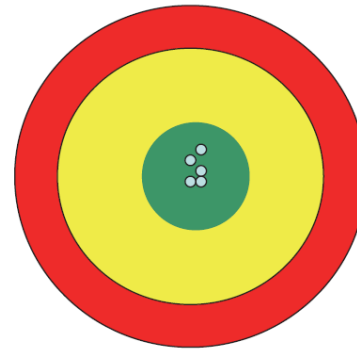
Precise and Accurate
IQC OK
EQA OK

2010 Izquierdo Álvarez et al "Procedures for Validation of Diagnostic Methods in Clinical Laboratory Accredited by ISO 15189" in Modern Approaches To Quality Control 2010

Case: Verification of kit and platform for NFL testing at Wieslab Diagnostic Services

- Kit: SIMOA® NF-light Advantage Kit for HD-1/HD-X
- Kit labelled as: For research use only. Not for use in diagnostic procedures!
- Analyser: Quanterix Simoa HD-X. CSV performed in Q1 2020
- Verification performed in Q1-Q3 2020 as three partial validations with the focus on the purpose of SVAR Diagnostic Services Laboratory

- Verification parameters and acceptance criteria established in collaboration with stakeholder: A physician specialized in clinical immunology laboratory as well as neurology
- Test not yet assigned "accredited at SVAR Wieslab Diagnostic Laboratory" since no external quality assurance programs exist
- Report of results from non-accredited tests acceptable according to Swedish ISO15189 inspectors
- Sponsors that are interested to use this assay as a biomarker are asked to consider additional partial validation for their context of use

Neurofilaments as a diagnostic marker

- Neurofilaments (NF) are useful as markers of acute and chronic neuronal injury (*Bacioglu M et al, 2016*)

- NFs are components of the neuronal cytoskeleton and are composed of three subunits:
  - neurofilament light (NFL), 68 kDA
  - neurofilament medium (NFM), 150 kDA
  - neurofilament heavy (NFH), 200 kDA

- NFL has been used as a diagnostic neuronal biomarker in CSF in Sweden since late 1990s (*Rosengren et al 1996*)

- Serum NFL levels and/or CSF NFL are increased in patients suffering from acute brain damage or chronic neurological disorders (*Bjornevik et al. 2019; Gil-Perotin et al. 2019; Rohrer et al. 2016; Rojas et al. 2016*)

"NFL - the C-Reactive Protein of Neurology"

Serum NFL has been found to be changed in most disorders of the central nerve system

- ✓ Multiple Sclerosis
- ✓ Amyotrophic Lateral Sclerosis
- ✓ Alzheimer's Disease
- ✓ Huntington's Disease
- ✓ Neuropathies
- ✓ Parkinson's Disease and Parkinsonian Disorders
- ✓ Stroke; AIS, TIA and HS, Small Vessel Disease, SAH
- ✓ Traumatic Axonal Injury; Severe TBI, Mild TI, Sport related Concussion
- ✓ Spinal Cord Injury
- ✓ Cardiac Arrest

Lambertsen et al. Brain Sci. 2020, 10, 56

Verification parameters and results of NFL

Parameter	Test and Result
Specificity/ Sensitivity	➢ Normal individuals (n=166) mixed ethnicity, gender and age confirmed normal ranges in serum published by Hviid CVB et al, 2020 ➢ Matched serum and EDTA-plasma (n=22 healthy and n=12 MS-patients) showed acceptable correlation between plasma/serum (R^2 0.86) ➢ CSF samples (n=25) from mixed ages with suspicion of neurological impairment was tested in a inter lab comparison with a good correlation (R^2 0.97)
Accuracy	➢ Tested as part of Specificity/Sensitivity
Precision	➢ Repeatability of 2 patient samples tested in 5 repetitions in 3 runs. ➢ Intra-assay precision: 4-9%CV ➢ Inter-assay precision: 9%CV ➢ Confirmed data from Quanterix (4-9%CV) and the acceptance criteria defined *a priori* (CV ≤ 20%)
Dilutional linearity (Parallelism)	➢ CSF: 1 sample tested and dilution up to 200x accepted (rRE< +/-20%) ➢ Serum: 1 sample tested and dilution up to 8x accepted (rRE< +/-20%) ➢ Confirmed Quanterix range 0.003–0.079 pg/mL in serum/plasma
Stability	➢ Freeze-Thaw ➢ Short Term Stability including bench top stability in room temperature ➢ Long term stability not performed due to diagnostic testing required with 1 week

Do a diagnostic lab always establish positive predictive value and negative predictive value?

- The clinical specificity and sensitivity is used to establish the positive predictive value (PPV) and negative predictive value (NPV)

- $PPV = \dfrac{Number\ of\ True\ Positives}{Number\ of\ True\ Positives + Number\ of\ False\ Positives}$

- $NPV = \dfrac{Number\ of\ True\ Negatives}{Number\ of\ True\ Negatives + Number\ of\ False\ Negatives}$

- In rare diseases it is not always possible to establish PPV and NPV due to limited number of reference samples

- In addition for some disease the analyte can be used in many different ways

- In these diseases the specificity of the test in the normal population and establishment of a normal range for a quantitative marker or a cut-point for an autoantibody tests is more relevant

- Important that individuals are not misdiagnosed. Specificity should be close to 100%

Is all tests performed by an ISO15189 lab not accredited?

- The majority of tests offered on the test menu should be accredited
- All tests should be validated/verified for its intended use
- An accredited test shall include both IQC and EQA continuous assay performance indicators
- A list of accredited tests offered should be easily retrievable for the customer for example on the web page of the laboratory
- Tests that are non-accredited are allowed and these are not allowed to be reported with the accreditation symbol on the test report

How do you control batch to batch variation of diagnostic assays?

- For accredited methods the IQC and EQA are used to control kit performance
  - IQC by Levy-Jennings charts
  - EQA for inter lab comparisons

- For non-accredited methods the most common control is to send your tests samples to another laboratory and perform comparison of test results
  - Results should give the correct diagnosis and for quantitative results within the established precision of your tests method

Can IVD/CE labelled kits be used in clinical trial (for example as a PD marker)?

- Yes – there is nothing in the IVD directives that inhibit a user in using IVD kits from the intended use as a diagnostic tests

- Intended use is described in the kit insert

- Consider if the IVD kits intended use is relevant for you purposes

- Make additional validation if the intended use for the Biomarker do not include the context of use in your clinical trial

Summary Biomarkers and Diagnostic labs

- CLIA labs must be used for decision making for US patients
- ISO accredited medical labs can be used for non-US patients
- Diagnostic laboratories have a certified quality management system (QMS)
  - But must be audited to fulfil the requirements of GCP
- When a validated diagnostic method is used – consider the Context of Use for the assay, potential re-development and perform additional validation if needed
- Final questions:
  - Can we use inspiration from Diagnostic labs for validation of Biomarker assays for the context of use?
  - Can diagnostic assay performance indicators (IQC, Levy-Jennings, Westgard, EQA) be used for biomarker assays?

Acknowledgements

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- SVAR Life Science Wieslab Diagnostic Services Laboratory
- Lotta Dahle at Linköping University Hospital
- Neurologists in Sweden for all the discussions of relevance of neurological biomarkers

QUESTIONS?
WE'D LOVE TO HEAR FROM YOU

Svar means answers in Swedish. If you have questions, we can help you find the right answers. Explore our website on www.svarlifescience.com