Performance Guide

iCheck
Fluoro
The test kit to measure vitamin A

BioAnalyt

Performance explained

This Performance Guide is intended to explain the validity of our test kit iCheck Fluoro in a nutshell to facilitate your decision making.

Complex language and processes are used during performance evaluation of a test kit, referred to as validation.

To clarify and harmonize this terminology and processes we have summarized how we validate our test kit and what the validation results mean.

We hope you find this material helpful and we are happy to receive your questions and comments! Do not hesitate to contact us at **support@bioanalyt.com.**

Sincerely,
Your BioAnalyt Support team

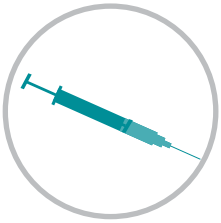
Development, manufacture and sales of all BioAnalyt test kits (devices, reagent vials) are carried out in accor-dance with ISO 9001:2015 and have been certified by TÜV NORD, Germany.

Note:
This material is based on the definitions set by ISO, the International Organization for Standardization in ISO 5725:1994.

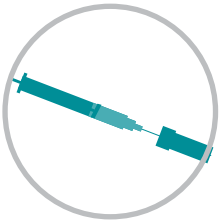
What is iCheck Fluoro?

iCheck Fluoro is an all-inclusive test kit for rapid on-the-spot measurement of vitamin A in food and biological fluids.

This test kit brings complex laboratory measurements down to a simple three-step process:



- Take up your sample

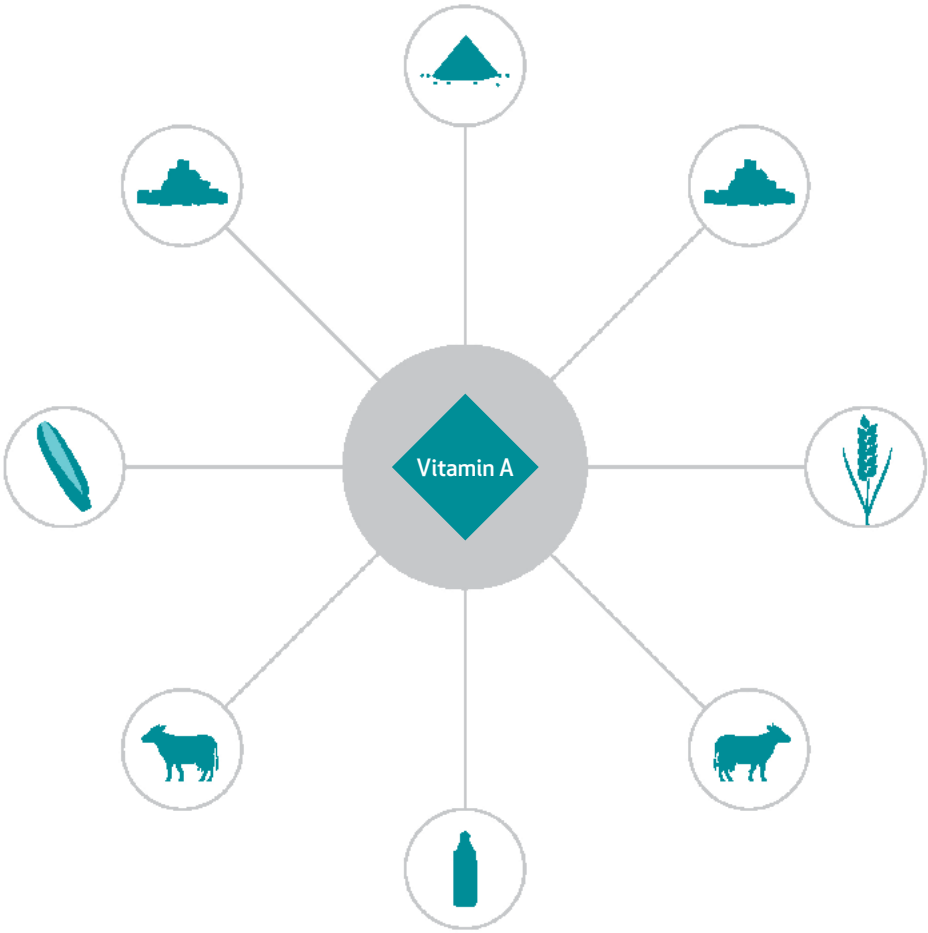


- Inject it into the ready-to-use reagent vial

- Measure the vial in your iCheck

What does iCheck Fluoro do?

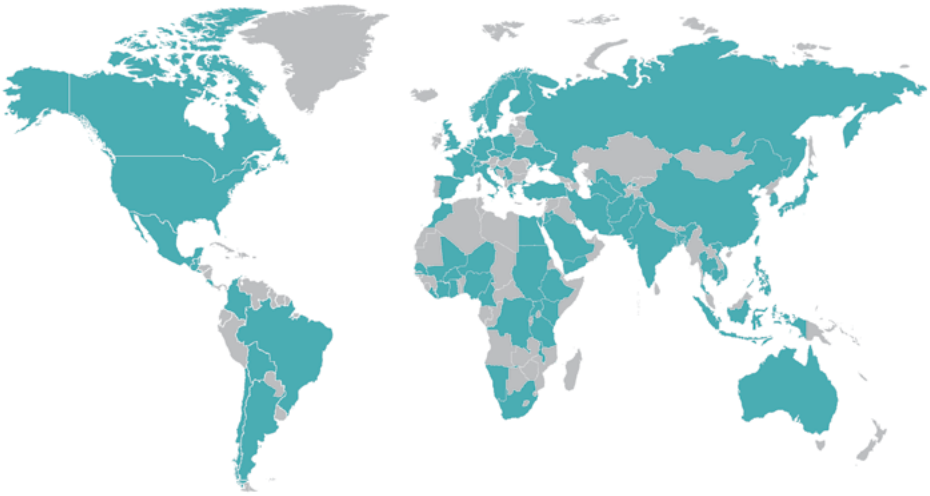
iCheck Fluoro measures vitamin A as retinyl palmitate , retinyl acetate and retinol in vitamin premix, sugar, flour, milk and breast milk.



Where are iChecks used?

iChecks are used in over 80 countries across the globe.

Our customers are leading international organizations such as UNICEF, World Food Program, Hellen Keller International , Global Alliance for Improved Nutrition (GAIN), ministries and monitoring agencies, micronutrient premix producers, academic institutions, and global and local food producers.

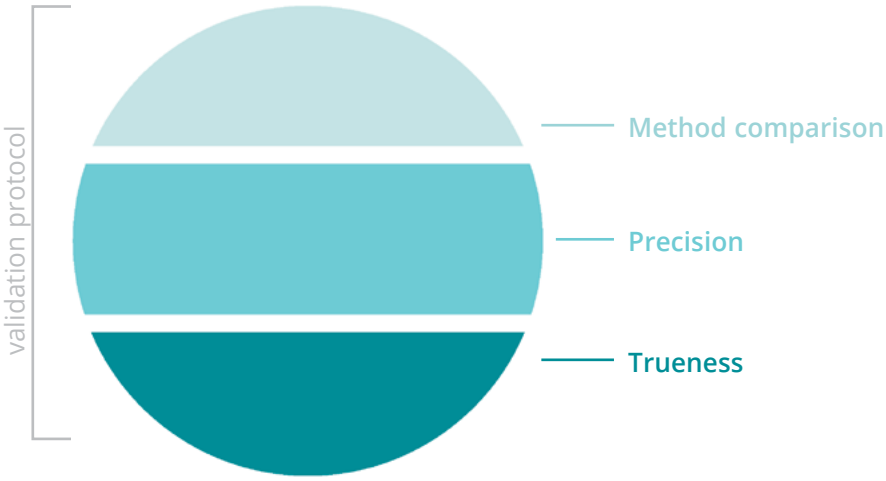


● Countries using iCheck

Is iCheck Fluoro validated?

We assess the performance of each test kit following a rigorous standardized process. This process is called a validation protocol.

The validation protocol combines assessment of precision, trueness and a comparison to a reference method.



How is iCheck Fluoro validated?

1 Assessment of precision

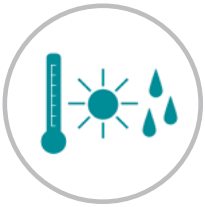
During the validation we assess the precision of the test kit. Precision tells us how similar are the repeated measurements of the same sample.

The difference observed between the repeated measurements is called variability of the result. The variability is common to all measurement methods and can be smaller or bigger.

Factors that affect the size of the variability of the result are:

- the sample itself

- the analyst



- the environment

- the instrument

We assess the precision by repeated measurements of the same sample under different conditions and by different people across the entire measurement range of iCheck Fluoro.

We have assessed the precision of vitamin A measurement with iCheck Fluoro for the following sample types: vitamin A standard, vitamin premix, flour, milk and sugar.

The variability observed between the measurements is defined as the coefficient of variation (CV). CV is calculated by dividing the standard deviation with the mean of the repeated measurements.

The CV for repeated measurements with iCheck Fluoro ranges from 3% to 15% depending on the type of the sample.

How is iCheck Fluoro validated?

2 Assessment of trueness

To know how close the measured result is to the real concentration of vitamin A we assess the trueness of the measurement.

To do this we add a known concentration of vitamin A to different types of samples and compare the expected concentration to the measured concentration.

The vitamin A that we use as a standard is a certified reference material which guarantees that our expected concentration is correct.

The trueness of iCheck Fluoro results is between 99% and 104% depending on the sample type. This means that of the expected 100% iCheck Fluoro measures 99 – 104% of the true vitamin A concentration in the sample.

The difference between expected and measured result is called bias and for iCheck Fluoro results it is at 1-4%.

Certain samples, such as flour have a matrix effect. We call matrix effect the value that iCheck Fluoro displays when we measure flour without any vitamin A added. For wheat flour the average matrix effect value is 0.65 mg RE/kg*.

This value is then deducted from the results with added vitamin A for the calculation of the real vitamin A content.

For more detailed information about the matrix effect and iCheck Fluoro measurement please contact as at ***support@bioanalyt.com***.

*RE: retinol equivalent is a unit for quantifying the vitamin A value of sources of vitamin A. RE is defined as 3.3 International Units (IU) of vitamin A or as 1 microgram of retinol.

3 Method comparison

We further assess the performance of our test kit against an accepted reference method.

For iCheck Fluoro the reference method is HPLC (high performance liquid chromatography). HPLC is a standard laboratory quantitative method for vitamin A measurement in food as described in Codex Alimentarius issued by World Health Organization and Food & Agriculture Organization.

We measure in parallel the same samples with iCheck and with HPLC and evaluate how do the results agree with one another.

The method comparison shows us that 98% of iCheck Fluoro results are in agreement with HPLC results. The results differ by a maximum of 6 mg RE/kg and on average by as low as 0.09 mg RE/kg*.

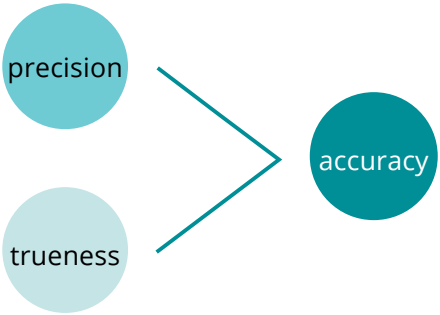
iCheck Fluoro HPLC

*Reference: Food fortification and vitamin A analysis. Andriette de Jaeger, Specialist Analyst, South African Grain Laboratory (SAGL). FST Magazine, May 2014.

How accurate are iCheck Fluoro results?

What is accuracy?
The assessment of the test kit's performance allows us to define how accurate iCheck Fluoro results are.

Accuracy combines both the precision and the trueness assessed during validation.



How do we calculate accuracy?
We express the accuracy of iCheck Fluoro in terms of the uncertainty of measurement.

To calculate this uncertainty we take the coefficient of variation (CV) associated with precision and the bias associated with trueness and combine them using the following equation:

- Uncertainty = bias + 1.96 x CV

This equation gives us the uncertainty of measurement based on all our observations during the validation. The uncertainty of measurement gives a range to the result and a 95% confidence level that the true value lies within that range.

The uncertainty of measurement with iCheck Fluoro for different sample types is listed in the table below.

SAMPLE TYPE	TRUENESS	BIAS	CV	UNCERTAINTY OF MEASUREMENT
Vitamin A Standard	102%	2%	5%	**10%**
Vitamin Premix	99%	1%	9%	**19%**
Sugar	103%	3%	5%	**13%**
Milk	104%	4%	3%	**10%**
Flour	100%	0%	15%	**30%**

Is this an acceptable accuracy?
It is important to note that the uncertainty does not imply doubt about the validity of a measurement. On the contrary, the knowledge of the uncertainty implies increased confidence in the validity of a measurement result.

The measurement of vitamin A in different foods and biological fluids using the reference method HPLC has the uncertainty between 5% and 30%, depending on the lab and the sample type. Similarly, the measurement with iCheck Fluoro has uncertainty between 10% and 30%.

The result you obtain using iCheck Fluoro has an accuracy level which enables you to make a confident decision.

What does the uncertainty mean to you?
You have measured your sugar sample and the result you have with the iCheck Fluoro is 2 mg/kg, after taking into account the dilution of solid sample in water.

The uncertainty of the iCheck Fluoro measurement is 13%. This means that the true concentration of vitamin A in your sugar sample is in the range of 2 mg/kg ± 13%.

The result is therefore documented in the following way:

- 2 ± 0.26 mg/kg or
- 1.74 – 2.26 mg/kg

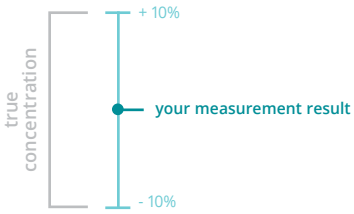
This range is then controlled against the required concentration.

How accurate are iCheck Fluoro results?

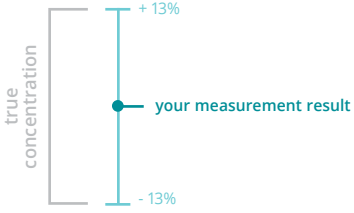
Accuracy and the sample type
The accuracy of your measurement depends to a large extent on the type of sample. Homogenous, in other words well-mixed samples such as vitamin A standards and milk have measurement uncertainty as low as 10%.

On the other hand dry solid samples such as flour have lower homogeneity. This leads to higher uncertainty of up to 30%.

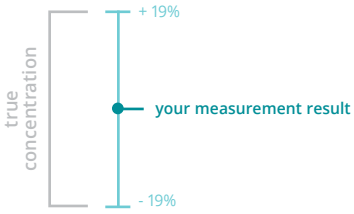
Vitamin A Standard and Milk



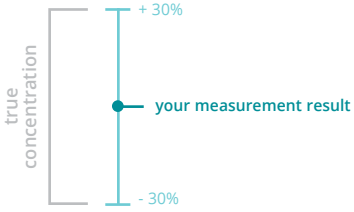
Sugar



Vitamin Premix



Flour



Technical Data

The technical specifications for iCheck Fluoro are listed in the table to the right for your reference. The measurement range of iCheck Fluoro is from 50 µg RE/L to 3000 µg RE/L. This corresponds to 0.05 to 3.0 mg/kg of vitamin A. If you would like more detailed information please contact us at **support@bioanalyt.com**.

TECHNICAL DATA	
Sample	
Analyte:	Vitamin A (retinol) as retinyl palmitate, retinyl acetate and other esters
Sample:	Food: premix, liquid milk, milk powder, flour, sugar, bouillon powder; biological fluids: breast milk, cattle whole blood and serum
Sample preparation:	For solid samples: dilution and homogenization in distilled or bottled water
Sample volume per analysis:	0.5 mL (500 µL)
Concentration range:	>0.05 ppm (mg/kg), samples above 3.0 ppm must be diluted in water
Device	
Analytical method:	Fluorimetric determination of retinol concentration using ultraviolate (UV) excitation
Units displayed:	µg RE/L, RE – retinol equivalents, µg - microgramms
Linear range:	50 – 3000 µg RE/L
Calibration:	Factory set (standards included for control)
Time per analysis:	< 10 min
Environment:	20 –30°C, no direct sunlight
Accuracy:	Coefficient of variation is 3 - 15%; extended measurement uncertainty at 95% confidence at 25 °C is 10 - 30% depending on sample type.
Method comparison:	High-performance liquid chromatography (HPLC)
User training:	1 day training
Use:	Laboratory and field
Data output:	Sample #, Batch #, Result, Date, Time (in transferred data)
Connectivity and data:	Results are stored in the device and transferred to a PC via USB
Power source:	NiMH rechargeable batteries included; AA 1.2 or 1.5V
Warranty:	2 years
Device weight:	0.45 kg
Device dimensions:	11 x 4 x 20 cm (W x H x L)
Test Kit	
Content:	100 reagent vials; 100 syringes - 1.0 mL; 100 needles - 1.6mm x 25mm.
Chemical composition:	n-Hexan and alcohols
Volume per reagent vial:	2.0 mL
Shelf life:	12 months at 20 –30°C, no direct sunlight, upright
Dimension of test kit:	26 x 14.5 x 16.5 cm
Disposal instructions:	Hazardous waste
Optional equipment:	Manual centrifuge, 50 mL falcons, weighing dishes, reference samples

Glossary
of the terms used

Accuracy	*closeness of an analytic result to an actual result. It is used to refer to both trueness and precision.*
Bias	*difference between expected and measured result due to systematic error of the measurement.*
Codex Alimentarius	*harmonized international food standards, guidelines and codes of practice to protect the health of the consumers and ensure fair practices in the food trade. It was established and is maintained by Food & Agriculture Organization (FAO) and World Health Organization (WHO).*
CV	*Coefficient of Variation. It is calculated by dividing the standard deviation with the mean of your repeated measurements. CV = Standard deviation / Mean x 100%. CV is observed due to random errors of the measurement.*
Dilution	*mixing of dry solid sample with water to bring the solid sample into the liquid state for injection into the reagent vial. Dilution is also recommended with concentrated liquid samples to fit to the measurement range of iCheck Fluoro. To get the concentration of vitamin A in the solid or concentrated sample the iCheck Fluoro result with diluted sample is multiplied with the dilution factor. The dilution factor is calculated by dividing the volume into which the sample was diluted with the weight of the solid or concentrated sample.*
Homogeneity	*uniformity of a substance or a uniform distribution of one substance within the other.*
HPLC	*High Performance Liquid Chromatography. It is one of the standard laboratory quantitative method for vitamin A measurement that involves extraction, separation and detection of the target analyte.*
ISO	*International Organization for Standardization*
Matrix effect	*interference stemming from unfortified food matrix. iCheck Fluoro measures the background fluorescence and displays a value with samples such as wheat and maize flour, despite no vitamin A present.*
Mean	*an average value of a set of values. It is calculated by summing up the values and dividing the sum with the number of values.*
Precision	*the extent to which a measurement procedure gives the same results each time it is repeated under identical conditions (repeatability) and variable conditions (reproducibility).*
RE	*Retinol Equivalent. It is a unit used for quantifying the vitamin A value of sources of vitamin A. RE is defined as 1 microgram of retinol.*
Retinol	*chemical name of vitamin A.*
Standard deviation	*a measure of the amount by which each value deviates from the mean of all values.*
Trueness	*closeness of agreement between the arithmetic mean of a large number of test results and the true or accepted reference value.*
Uncertainty of measurement	*the doubt that exists about the result of any measurement. It combines the random error (CV) and the systematic error (bias) following the equation: uncertainty = bias + 1.96 x CV. The uncertainty of measurement gives the result a range and there is 95% confidence that the true value lies within that range.*
Validation	*an analytical procedure performed with the objective to demonstrate that the analytical method is suitable for its intended purpose. During validation accuracy, precision, trueness, specificity and sensitivity of the analytical method for a certain analyte in a certain matrix is assessed.*
Variability	*a measure of the spread of a set of values from the reference or the mean value.*

Quality Guarantee

iCheck is produced following strict rules of quality assurance according to ISO 9001:2015. This is accomplished by the use of high-grade components and equipment as well as a stream-lined production process. This process includes quality controls for each component and rigorous calibration of the device by trained technicians.

Your iCheck Fluoro comes with a 2-year warranty.

If you have any questions, please contact us by calling **+49 (0)33 28 35 15 000** or sending an e-mail to **support@bioanalyt.com.**

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This material has been developed with support from Global Alliance for Improved Nutrition.

"A partnership to improve the quality of nutritious foods".

BioAnalyt

measure for life

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