

# iCheck Fluoro

## Measuring Vitamin A in Animal Liver

### 1. Method Principle and Application

iCheck Fluoro is a portable fluorometer for quantitative determination of the vitamin A content in a variety of samples, including flour, sugar, premix, animal blood, liver and dairy. The method is based on the fluorescence of the retinol molecule (excitation at 340 nm, emission at  $\geq 400$  nm). The device algorithm calculates vitamin A concentration in  $\mu\text{g}$  retinol equivalents (RE) per liter ( $\mu\text{g RE/L}$ ). The measurement range of iCheck Fluoro is 50 – 3000  $\mu\text{g RE/L}$ .



### 2. Animal Liver as Source of Vitamin A in Pet Food

Liver is among the richest sources of vitamin A in pet food. Vitamin A in liver is primarily found in the form of retinyl esters (mostly retinyl palmitate), making it highly bioavailable. While vitamin A is necessary for animal development and health maintenance, it can also lead to toxicity if supplied in excess.

Vitamin A levels in liver vary widely even within a single species (Table 1).

**Table 1. Vitamin A levels in beef, pork and chicken liver**

Sample Type	Liver vitamin A levels [mg RE/kg]
Beef liver	16 – 260 <sup>[1]</sup>
Pork liver	65 – 189 <sup>[2]</sup>
Chicken liver	16 – 166 <sup>[2]</sup>

Hence, it is crucial to regularly monitor vitamin A levels in animal liver used in pet food production to prevent toxicity and ensure consistent product quality. Recommended vitamin A concentration in pet food depends on the species and life stage (e.g., puppy/kitten vs. adult). Generally, the minimum recommended amount for cats and dogs is 5000 IU/1000 kcal.

### 3. iCheck Fluoro Performance with Animal Liver

iCheck Fluoro performance is assessed following a standardized process that combines assessment of precision, trueness and a comparison to a reference method (i.e., high-performance liquid chromatography – HPLC). The detailed description of this process is provided in the [iCheck Fluoro Performance Guide](#).

Performance of iCheck Fluoro with pork, beef and chicken liver has been assessed in internal validations and compared to HPLC. Below is a table showing the results obtained with both methods.

**Table 2. Vitamin A quantification in animal liver with iCheck Fluoro and HPLC**

Sample Type	iCheck Fluoro [IU/kg]	Coefficient of variation of iCheck	HPLC [IU/kg]	Recovery: iCheck Fluoro vs. HPLC
Beef liver	870,000	4%	720,000	121%
Pork liver	446,666	10%	393,333	114%
Chicken liver	316,666	13%	313,333	101%

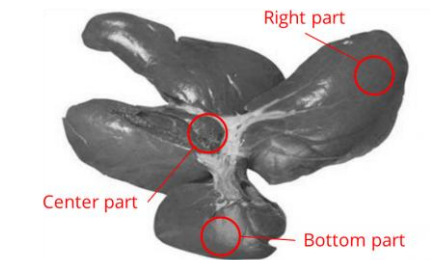
### 4. Analyzing Vitamin A in Animal Liver with iCheck Fluoro

#### Sampling:

Due to high variability of vitamin A content both between and within livers, appropriate sampling is crucial. The sample size should be proportional to the total amount of liver the sample represents.

#### Determining sample size:

- In industrial settings, large sample sizes are typically required due to high production volumes, i.e., total liver amounts range from several hundred kilograms to several tonnes. In this case, the representative sample should be approximately 3-5 kg.
- If the testing is done on a single liver, for most representative results, the entire liver should be used. Alternatively, obtain portions from the bottom, center and right parts of the liver (Fig. 1).



**Figure 1. Parts of the liver to be sampled**



### Sample homogenization:

Once you have obtained your representative sample, proceed with sample preparation.

- If frozen, thaw your sample.
- First, grind the sample using a meat grinder (Fig.2).
- Then, transfer the sample to a blender (laboratory or kitchen) (Fig.2) and process it for 1 min. After this, your sample should appear as a sticky uniform paste.

**A**



**B**



**Figure 2. A: meat grinder; B: kitchen blender**

### Sample Dilution:

- Weigh in 0.2 g of your well-mixed homogenized liver sample into a 50 mL Falcon tube. Record the exact weight for dilution factor calculation.
- Add bottled or distilled water to a total volume of 20 mL.
- Shake the solution vigorously by hand for 1 minute.
- Do not let the sample settle, immediately proceed with taking the sample suspension up into the syringe and injecting it into the iCheck Fluoro reagent vial and proceed as described in the [iCheck Fluoro User Manual](#).



## 6. Dilution Factor (DF) Calculation

The value displayed on the iCheck Fluoro after measurement will reflect the concentration of vitamin A in the diluted sample. To obtain the original liver vitamin A concentration, you must first calculate the dilution factor according to the following formula:

$$DF = \frac{\text{Total sample solution volume (mL)}}{\text{Sample weight (g)}}$$

Once you have calculated the dilution factor, multiply the iCheck Fluoro result by the dilution factor:

$$\text{Vitamin A in Liver} \left( \frac{\text{mg RE}}{\text{kg}} \right) = \text{iCheck Fluoro result} \left( \frac{\mu\text{g RE}}{\text{L}} \right) \times DF / 1000$$

## 7. Vitamin A Unit Conversion

Below you can find the relationship between retinol equivalents and other units used for vitamin A quantification.

- **1 mg vitamin A = 1 mg retinol = 1 mg RE**
- **1 mg RE = 3333 International Units (IU)**
- **0.3 IU = 0.001 mg RE = 1 g RE**
- **1 µg RE = 3.333 IU**
- **1 mg = 1000 µg**

*Example:*

$$65 \text{ mg RE/kg} / (0.3/1000) = 216,667 \text{ IU/kg}$$

[1] Schulz K., et al. Vitamin A concentration in bovine liver and milk does not only depend on characteristics of the farming system. NPJ Sci Food. 2025 Mar 17;9(1):32

[2] Majchrzak D. et al. Vitamin A content (retinol and retinyl esters) in livers of different animals, Food Chemistry, Volume 98, Issue 4, 2006, Pages 704-710.

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