



## **Final Report**

evaluation of proficiency test

### **DLA ptAUS1 (2025)**

## **Animal Species-Screening I - 3 Samples qualitative (with known contents):**

**Beef, Goat, Poultry (Chicken and Duck (genus:  
*anas* or *cairina*)) and Sheep**

**in Cooked Meat Product (Sausage Meat)**

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**General Information on the proficiency test (PT)**

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<i>Vertraulichkeit</i> <i>Confidentiality</i>	Die Teilnehmerergebnisse sind im EP-Bericht in anonymisierter Form mit Auswertenummern benannt. Daten einzelner Teilnehmer werden ausschließlich nach vorheriger Zustimmung des Teilnehmers an Dritte weitergegeben. Participant results are named anonymously with evaluation numbers in the PT report. Data of individual participants will be passed on to third parties only with prior consent of the participant.

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## 1. Introduction

The participation in proficiency test (PT) schemes is an essential element of the quality management system of every laboratory testing food and feed, cosmetics and food contact materials. The implementation of proficiency tests enables the participating laboratories to prove their own analytical competence under realistic conditions. At the same time they receive valuable data regarding the verification and/or validation of the particular testing method [1, 5].

The purpose of DLA is to offer proficiency tests for selected parameters in concentrations with practical relevance.

Realisation and evaluation of the present proficiency test follows the technical requirements of DIN EN ISO/IEC 17043 (2010) and DIN ISO 13528:2022 [2, 3].

The general procedure for evaluating the DLA proficiency tests can be found in the “**DLA Evaluation Guide 02.02 (2024) General Proficiency Test Schemes**”.

## 2. Realisation

### 2.1 Test material

Three different PT samples with possible contents of animal foods of beef, goat, poultry (chicken and duck (genus: *anas* or *cairina*)) and sheep were provided for qualitative determination. The parameters were present in the matrix cooked meat product (sausage meat) with contents of 1-100%.

#### Preparation of the samples:

The respective raw materials for the animal species used were commercially available meat products.

The raw materials (meat species) have been minced separately.

By using a meat cutter, the corresponding quantitative amounts of raw materials for each sample (see table 2) and the further ingredients (see Table 1) were homogenized and a sausage meat was produced. After homogenization, the sausage meat was filled into portions of approx. 30 g in plastic containers and then heated for one hour at 100 °C in a water bath.

**Table 1: Composition of DLA-Samples**

Ingredients	Samples 1 - 3
Total meat content	64 - 72 %
Water	23 - 30 %
Gelatine (100% pork)	3,0 - 3,5 %
Sodium chloride	0,8 – 2,2 %
Sorbic acid	0,33 – 0,39 %

**Table 2:** Contents (in % of total weight) of the respective animal species in the sausage meat samples 1-3.


Ingredients *	Sample 1	Sample 2	Sample 3
Beef meat	positive (3,9%)	negative	positive (49%)
Duck meat (genus: <i>anas</i> )	negative	positive (6,6%)	positive (8,6%)
Duck meat (genus: <i>cairina</i> )	negative	negative	negative
Chicken meat	positive (49%)	positive (16%)	negative
Goat meat	positive (18%)	negative	positive (7,7%)
Sheep meat	negative	positive (43%)	positive (6,3%)
further ingredients from animal species which were not requested in this proficiency test round:			
Pork gelatine	positive (3,2%)	positive (3,5%)	positive (2,9%)

\* Animal species contents of „food item“ as indicated in the column of ingredients according gravimetric mixing

**Note:** The metrological traceability of temperature, mass and volume during production of the PT samples is ensured by DAkkS-compliant calibrated reference materials.

All samples were analysed by an external laboratory using the SureFood® ANIMAL ID 4plex Beef/Sheep/Goat+IAAC, SureFood® ANIMAL ID Chicken IAAC, in-house method Animal ID Duck IAAC and SureFood® ANIMAL ID Pork SENS PLUS PCR methods from CONGEN (Congen Biotechnologie GmbH, Germany). The results are mostly consistent with the animal species added to samples 1-3 (compare Tables 2 and 3). One exception is sample 1, in which DNA from sheep was detected, even though no sheep meat was added to this sample.

**Table 3:** Verification of detectability of the animal species in samples 1-3 by Real Time PCR.

	SureFood® ANIMAL ID 4plex Beef/Sheep/Goat+IAAC*		
	SureFood® ANIMAL ID Chicken IAAC**		
	SureFood® ANIMAL ID Pork SENS PLUS***		
	and in-house method Animal ID Duck IAAC****		
	Sample 1	Sample 2	Sample 3
Beef	positive	-	positive
Duck	-	positive	positive
Chicken	positive	positive	-
Goat	positive	-	positive
Sheep	positive	positive	positive
Pork (from 100% pork gelatine)	positive	positive	positive

\* Limit of detection (LOD) 0,01 % (w/w); \*\* LOD 0,1% (w/w); \*\*\* LOD 0,0001% (w/w); \*\*\*\* LOD not available

## **2.1 Stability**

The sample material is sausage meat, which contains sorbic acid and has been heated to 100°C for 1 h after production and bottling. The storage stability or shelf life of the samples (microbial spoilage) is thus guaranteed during the examination period under the specified storage conditions.

## **2.2 Sample shipment and information to the test**

The portions of the test materials (samples 1 to 3) were sent to every participating laboratory on 2025-03-19. The testing method was optional. The tests should be finished at 2025-05-02 the latest. With the cover letter along with the sample shipment, the following information was given to the participants:

*There are 3 different samples possibly containing the animal products Beef, Goat, Poultry (Chicken and Duck (genus: anas or cairina)) and Sheep. The parameters are contained in the matrix of cooked Meat Product (Sausage Meat) with amounts of 1 – 90%. The evaluation of results is strictly qualitative (positive / negative).*

Note: Samples should be stored at 2-10 °C upon arrival.

Before analysis, the entire sample quantity should be homogenized, since components such as fat can separate during the production/processing of the samples.

**Please note the attached information on the proficiency test.**

(see documentation, section 5.2 Information on the PT)

## **2.3 Submission of results**

The participants submitted their results online via an internet portal (my DLA | participant's portal at <https://my.dla-pt.com>) filled in standardized tables. The results given as positive/negative were evaluated. Queried and documented were the indicated results and details of the test methods like specificities, test kit manufacturer and hints about the procedure.

In case participants submitted several results for the same parameter obtained by different methods, these results were evaluated with the same evaluation number with a letter as a suffix and indication of the related method.

All 24 participants submitted at least one result.

A registration was canceled before the samples were sent.

### 3. Evaluation

Evaluation of the qualitative animal species-screenings is done separately for the following groups of methods: protein-based methods (e.g. ELISA, IEF, MALDI-TOF) and DNA-based methods (e.g. PCR-techniques, NGS, Microarrays).

Different protein and DNA-based methods for the determination of animal species in foods are eventually using different antibodies and target-DNA, are usually calibrated with different reference materials and may utilize differing extraction methods. Among others this can induce different valuation of the presence and/or content of the analyte. Furthermore, matrix and/or processing (especially by the use of ELISA methods) as well as the type of meat component used (musculature or internal organs such as liver) can strongly influence the detectability of animal species [A].

#### **3.1 Agreement with Consensus Values from Participants**

The qualitative evaluation of results of each participant is based on the agreement of the indicated results (positive or negative) with the **consensus values from participants**. A consensus value is determined if  $\geq 75\%$  positive or negative results are available for a parameter.

The assessment will be in the form that the number of matching results followed by the number of samples for which a consensus value was obtained is indicated. Behind that the agreement is expressed as the percentage in parentheses. For interpretation of the valuation see Table 4. In all cases, it is recommended to compare the agreement with the consensus values from participants with the agreement of results with the spiking of samples (3.2).

#### **3.2 Agreement with the Spiking of Samples**

The qualitative evaluation of the results of each participant is based on the agreement of the indicated results (positive or negative) with the **spiking of the three PT-samples**.

The assessment will be in the form that the number of matching results followed by the number of samples is indicated. Behind that the agreement is expressed as the percentage in parentheses.

**Table 4: Qualitative evaluation** of participant results for the 3 animal-species screening samples

Agreement with consensus values	Interpretation (valuation)
3/3 results (100%)	All 3 samples were assigned correctly ( <b>successful</b> )
2/3 results (67%), or 1/3 results (33%)	<p>1 or more of the 3 samples were assigned <b>“falsely” negative</b></p> <p><b>a)</b> no positive sample was detected (<b>not successful</b>)</p> <p><b>b)</b> one or more of the positive samples were not detected.</p> <p>Check the LOQ/LOD of the applied method against the spiking level of the parameter as given in table 2 (added animal species):</p> <p>The spiking level of the “false” negative sample is near or below the LOD/LOQ, which should be suitable for the purpose of animal species levels (<b>can be acceptable</b>)</p> <p>The spiking level of the “false” negative sample is well above the LOD/LOQ, e.g. 3 times higher (<b>not plausible</b>)</p>
2/3 results (67%), or 1/3 results (33%)	<p>1 or more of the 3 samples were assigned <b>“falsely” positive</b></p> <p><b>c)</b> the “false” positive result is near or below the LOD/LOQ of the applied method, which should be suitable for the purpose of animal species levels (<b>can be acceptable</b>)</p> <p><b>d)</b> the “false” positive result is well above the LOD/LOQ, e.g. 3 times higher (<b>not plausible</b>),</p> <p><b>e)</b> check for cross-reactivities of the applied method against other animal species as given in table 2 (added animal species). Known cross-reactivities should be routinely considered for the respective analysis (<b>can be acceptable</b>)</p>
0/3 results (0%)	All samples were not assigned correctly ( <b>not successful</b> ): possibly some positive and negative samples were mixed up.



## 4. Results

All following tables are anonymized. With the delivering of the evaluation report, the participants are informed about their individual evaluation number.

The qualitative evaluation is done separately for the following groups of methods: protein-based methods (e.g. ELISA, IEF, MALDI-TOF) and DNA-based methods (e.g. PCR-techniques, NGS, Microarrays). Within these groups the results are sorted according to the applied sub-groups (e.g. test kits) and sorted chronologically according to the evaluation number of the participants.

The participant results and evaluation are tabulated as follows:

Evaluation number	Sample 1	Sample 2	Sample 3	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		

	Sample 1	Sample 2	Sample 3
Number positive			
Number negative			
Percent positive			
Percent negative			
Consensus value			
Spiking			

## 4.1 Proficiency Test Beef meat

### 4.1.1 Immunological protein-based methods (e.g. ELISA, IEF, MALDI-TOF)

#### Beef meat

#### Qualitative Valuation of Results

Evaluation number	Sample 1	Sample 2	Sample 3	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
6	positive	negative	positive	-	3/3 (100%)	ETM	

	Sample 1	Sample 2	Sample 3
Number positive	1	0	1
Number negative	0	1	0
Percent positive	100	0	100
Percent negative	0	100	0
Consensus value	-	-	-
Spiking	positive	negative	positive

#### Methods:

ETM = ELISA-TEK™ Cooked Meat Species Kits

#### Comments:

Only one participant submitted results obtained by protein-based methods (ELISA) for beef meat. The results for samples 1-3 are in qualitative agreement with the spiking of sample 1 (3,9% beef meat) and sample 3 (49% beef meat), as well as the consensus values as determined by the DNA-based methods (see 4.1.2).

### 4.1.2 Molecular biological DNA-based methods (e.g. PCR, NGS, Microarray)

#### Beef meat

#### Qualitative Valuation of Results

Evaluation number	Sample 1	Sample 2	Sample 3	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
9	positive	negative	positive	3/3 (100%)	3/3 (100%)	ASU	
4a	positive	negative	positive	3/3 (100%)	3/3 (100%)	ASU	
4b	positive	negative	positive	3/3 (100%)	3/3 (100%)	ASU	
17	positive	positive	positive	2/3 (67%)	2/3 (67%)	BP	
16	positive	positive	positive	2/3 (67%)	2/3 (67%)	FP-1	
23	positive	negative	positive	3/3 (100%)	3/3 (100%)	GI-T	
5	positive	negative	positive	3/3 (100%)	3/3 (100%)	GSD	
15	positive	positive	positive	2/3 (67%)	2/3 (67%)	GSD	
1	positive	negative	positive	3/3 (100%)	3/3 (100%)	ISO	
11a	positive	negative	positive	3/3 (100%)	3/3 (100%)	NGS	traces of cow DNA in sample 2 below LOD
2	positive	negative	positive	3/3 (100%)	3/3 (100%)	PCR	
3	positive	negative	positive	3/3 (100%)	3/3 (100%)	PCR	
8	positive	negative	positive	3/3 (100%)	3/3 (100%)	PCR	
13	positive	negative	positive	3/3 (100%)	3/3 (100%)	PCR	
14	positive	positive	positive	2/3 (67%)	2/3 (67%)	PCR	
18	positive	positive	positive	2/3 (67%)	2/3 (67%)	PCR	
19	positive	negative	positive	3/3 (100%)	3/3 (100%)	PCR	
24	positive	negative	positive	3/3 (100%)	3/3 (100%)	PCR	
11b	positive	negative	positive	3/3 (100%)	3/3 (100%)	PCR	traces of cow DNA in sample 2 below LOD
20a	positive	negative	positive	3/3 (100%)	3/3 (100%)	PCR	
22	positive	negative	positive	3/3 (100%)	3/3 (100%)	PCR / NGS	
6	positive	negative	positive	3/3 (100%)	3/3 (100%)	RF	
12	positive	negative	positive	3/3 (100%)	3/3 (100%)	RF	
10	positive	negative	positive	3/3 (100%)	3/3 (100%)	SFA-4p	
20b	positive	negative	positive	3/3 (100%)	3/3 (100%)	SFA-4p	

	Sample 1	Sample 2	Sample 3
Number positive	25	5	25
Number negative	0	20	0
Percent positive	100	20	100
Percent negative	0	80	0
Consensus value	positive	negative	positive
Spiking	positive	negative	positive

#### Methods:

ASU = ASU §64 method

BP = Bosphore Species Identification Kit, Anatolia Genew orks

FP-1 = foodproof® Animal Detection 1 LyoKit, Hygiena

GI-T = GEN-IAL First- Animal (Tetraplex PCR)

GSD = DNAimal Ident, Gold Standard Diagnostics

ISO = ISO method

NGS = house method NGS

PCR = house method PCR

RF= RapidFinder™ ID Kit, ThermoFisher

#### Comments:

The consensus values of the results of samples 1-3 are in qualitative agreement with the spiking of sample 1 (3,9% beef meat) and sample 3 (49% beef meat). Some participants reported positive results for beef in sample 2. No beef meat was added to the sample. However, traces of beef DNA cannot be excluded.

## **4.2 Proficiency Test Goat meat**

### **4.2.1 Immunological protein-based methods (e.g. ELISA, IEF, MALDI-TOF)**

#### **Goat meat**

#### **Qualitative Valuation of Results**

No results were submitted for the parameter goat using protein-based methods.

### 4.2.2 Molecular biological DNA-based methods (e.g. PCR, NGS, Microarray)

#### Goat meat

#### Qualitative Valuation of Results

Evaluation number	Sample 1	Sample 2	Sample 3	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
4	positive	negative	positive	3/3 (100%)	3/3 (100%)	ASU	
9	positive	positive	positive	2/3 (67%)	2/3 (67%)	ASU	
17	positive	negative	positive	3/3 (100%)	3/3 (100%)	BP	
23	positive	positive	positive	2/3 (67%)	2/3 (67%)	GI-S	
15	positive	negative	positive	3/3 (100%)	3/3 (100%)	GS	
5	positive	negative	positive	3/3 (100%)	3/3 (100%)	GSD	
21	positive	negative	positive	3/3 (100%)	3/3 (100%)	ISO	
11a	positive	negative	positive	3/3 (100%)	3/3 (100%)	NGS	traces of goat DNA in sample 2 below LOD
1	positive	negative	positive	3/3 (100%)	3/3 (100%)	PCR	
2	positive	negative	positive	3/3 (100%)	3/3 (100%)	PCR	
3	positive	positive	positive	2/3 (67%)	2/3 (67%)	PCR	
11b	positive	negative	positive	3/3 (100%)	3/3 (100%)	PCR	traces of goat DNA in sample 2 below LOD
13	positive	negative	positive	3/3 (100%)	3/3 (100%)	PCR	
14	positive	negative	positive	3/3 (100%)	3/3 (100%)	PCR	
16	positive	positive	positive	2/3 (67%)	2/3 (67%)	PCR	
18	positive	negative	positive	3/3 (100%)	3/3 (100%)	PCR	
19	positive	negative	positive	3/3 (100%)	3/3 (100%)	PCR	
20a	positive	negative	positive	3/3 (100%)	3/3 (100%)	PCR	
24	positive	negative	positive	3/3 (100%)	3/3 (100%)	PCR	
22	positive	negative	positive	3/3 (100%)	3/3 (100%)	PCR / NGS	
6	positive	negative	positive	3/3 (100%)	3/3 (100%)	RF	
12	positive	negative	positive	3/3 (100%)	3/3 (100%)	RF	
10	positive	negative		2/2 (100%)	2/2 (100%)	SFA-4p	
20b	positive	negative	positive	3/3 (100%)	3/3 (100%)	SFA-4p	

	Sample 1	Sample 2	Sample 3
Number positive	24	4	23
Number negative	0	20	0
Percent positive	100	17	100
Percent negative	0	83	0
Consensus value	positive	negative	positive
Spiking	positive	negative	positive

#### Methods:

ASU = ASU §64 method

BP = Bosphore Species Identification Kit, Anatolia Genew orks

GI-S = GEN-IAL First- Animal (single PCR)

GSD = DNAAnimal Ident, Gold Standard Diagnostics

GS = Eurofins Genescan DNAAnimal Ident

ISO = ISO method

NGS = house method NGS

PCR = house method PCR

RF= RapidFinder™ ID Kit, ThermoFisher

#### Comments:

The consensus values of the results of samples 1-3 are in qualitative agreement with the spiking of sample 1 (18% goat meat) and sample 3 (7,7% goat meat). Some participants reported positive results for goat in sample 2. No meat from goat was added to this sample, however, the sample contains large amounts of sheep meat (43%) and there could be cross-reactions using some methods due to the highly similar DNA sequences of these species. Traces of goat DNA cannot be excluded either.

## **4.3 Proficiency Test Poultry meat in general**

### **4.3.1 Immunological protein-based methods (e.g. ELISA, IEF, MALDI-TOF)**

#### **Poultry meat in general**

#### **Qualitative Valuation of Results**

No results were submitted for the parameter poultry using protein-based methods.

### 4.3.2 Molecular biological DNA-based methods (e.g. PCR, NGS, Microarray)

#### Poultry meat in general

#### Qualitative Valuation of Results

Evaluation number	Sample 1	Sample 2	Sample 3	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
16	positive	positive	positive	3/3 (100%)	3/3 (100%)	FP-2	The kit detects: chicken (Gallus gallus), turkey (Meleagris gallopavo) and poultry/aves (includes Anser, Anas, Struthio, Gallus, Meleagris, Numida & other birds. Poultry is able to be detected with this kit so far.
23	negative	negative	negative			GI-T	Results excluded / not plausible: Participant has obtained positive results for chicken in sample 1 and 2.
11a	positive	positive	positive	3/3 (100%)	3/3 (100%)	NGS	
1	positive	positive	positive	3/3 (100%)	3/3 (100%)	PCR	The result is given as presence of chicken and duck
18	positive	positive	negative	2/3 (67%)	2/3 (67%)	PCR	
11b	positive	positive	positive	3/3 (100%)	3/3 (100%)	PCR	
22	positive	positive	negative	2/2 (100%)	2/2 (100%)	PCR / NGS	Result for sample 3 excluded: Participant indicates that he can only detect chicken and turkey with his method.
6	positive	positive	positive	3/3 (100%)	3/3 (100%)	RF	
8	positive	positive		2/2 (100%)	2/2 (100%)	SFA-4p	

	Sample 1	Sample 2	Sample 3
Number positive	8	8	4
Number negative	0	0	1
Percent positive	100	100	80
Percent negative	0	0	20
Consensus value	positive	positive	positive
Spiking	positive	positive	positive

#### Methods:

FP-2 = foodproof® Animal Detection 2 LyoKit, Hygiena

GI-T = GEN-IAL First- Animal (Tetraplex PCR)

NGS = house method NGS

PCR = house method PCR

RF= RapidFinder™ ID Kit, ThermoFisher

SFA-4p = Sure Food Animal ID 4plex, R-Biopharm / Congen

#### Comments:

The consensus values of the results of samples 1-3 are in qualitative agreement with the spiking of sample 1 (49% chicken meat), sample 2 (16% chicken meat and 6% duck meat) and sample 3 (8,6% duck meat).

## **4.4 Proficiency Test Chicken meat**

### **4.4.1 Immunological protein-based methods (e.g. ELISA, IEF, MALDI-TOF)**

#### **Chicken meat**

#### **Qualitative Valuation of Results**

No results were submitted for the parameter chicken using protein-based methods.



#### 4.4.2 Molecular biological DNA-based methods (e.g PCR, NGS, Microarray)

##### Chicken meat

##### Qualitative Valuation of Results

Evaluation number	Sample 1	Sample 2	Sample 3	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
4a	positive	positive	negative	3/3 (100%)	3/3 (100%)	ASU	
4b	positive	positive	negative	3/3 (100%)	3/3 (100%)	ASU	
9	positive	positive	negative	3/3 (100%)	3/3 (100%)	ASU	
17	positive	positive	negative	3/3 (100%)	3/3 (100%)	BP	
16	positive	positive	negative	3/3 (100%)	3/3 (100%)	FP-2	
23	positive	positive	negative	3/3 (100%)	3/3 (100%)	GI-T	
5	positive	positive	negative	3/3 (100%)	3/3 (100%)	GS	
15	positive	positive	negative	3/3 (100%)	3/3 (100%)	GS	
21	positive	positive	negative	3/3 (100%)	3/3 (100%)	ISO	
11a	positive	positive	negative	3/3 (100%)	3/3 (100%)	NGS	
1	positive	positive	negative	3/3 (100%)	3/3 (100%)	PCR	
2	positive	positive	negative	3/3 (100%)	3/3 (100%)	PCR	traces of chicken DNA in sample 3 below LOD
3	positive	positive	negative	3/3 (100%)	3/3 (100%)	PCR	
7	positive	negative	negative	2/3 (67%)	2/3 (67%)	PCR	
11b	positive	positive	negative	3/3 (100%)	3/3 (100%)	PCR	
13	positive	positive	negative	3/3 (100%)	3/3 (100%)	PCR	
14	positive	positive	negative	3/3 (100%)	3/3 (100%)	PCR	
18	positive	positive	negative	3/3 (100%)	3/3 (100%)	PCR	
19	positive	positive	negative	3/3 (100%)	3/3 (100%)	PCR	
20a	positive	positive	negative	3/3 (100%)	3/3 (100%)	PCR	
24	positive	positive	negative	3/3 (100%)	3/3 (100%)	PCR	
22	positive	positive	negative	3/3 (100%)	3/3 (100%)	PCR / NGS	
6	positive	positive	negative	3/3 (100%)	3/3 (100%)	RF	
12	positive	positive	negative	3/3 (100%)	3/3 (100%)	RF	
8	positive	positive	negative	3/3 (100%)	3/3 (100%)	SFA-4p	
20b	positive	positive	negative	3/3 (100%)	3/3 (100%)	SFA-ID	

	Sample 1	Sample 2	Sample 3
Number positive	26	25	0
Number negative	0	1	26
Percent positive	100	96	0
Percent negative	0	4	100
Consensus value	positive	positive	negative
Spiking	positive	positive	negative

##### Methods:

ASU = ASU §64 method

BP = Bosphore Species Identification Kit, Anatolia Genew orks

FP-2 = foodproof® Animal Detection 2 LyoKit, Hygiena

GI-T = GEN-IAL First- Animal (Tetraplex PCR)

GS = Eurofins Genescan DNAAnimal Ident

ISO = ISO method

NGS = house method NGS

PCR = house method PCR

RF= RapidFinder™ ID Kit, ThermoFisher

SFA-4p = Sure Food Animal ID 4plex, R-Biopharm / Congen

SFA-ID= SureFood Animal ID, R-Biopharm / Congen

##### Comments:

The consensus values of the results of samples 1-3 are in qualitative agreement with the spiking of sample 1 (49% chicken meat) and sample 2 (16% chicken meat).

## **4.5 Proficiency Test Duck meat (in general and genus *anas* / *cairina*)**

### **4.5.1 Immunological protein-based methods (e.g. ELISA, IEF, MALDI-TOF)**

#### **Duck meat (in general)**

#### **Qualitative Valuation of Results**

No results were submitted for the parameter duck using protein-based methods.

#### 4.5.2 Molecular biological DNA-based methods (e.g. PCR, NGS, Microarray)

##### Duck meat in general

##### Qualitative Valuation of Results

Evaluation number	Sample 1	Sample 2	Sample 3	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
1	negative	positive	positive	3/3 (100%)	3/3 (100%)	PCR	
13	negative	positive	positive	3/3 (100%)	3/3 (100%)	PCR	
5	negative	positive	positive	3/3 (100%)	3/3 (100%)	GS	

	Sample 1	Sample 2	Sample 3
Number positive	0	3	3
Number negative	3	0	0
Percent positive	0	100	100
Percent negative	100	0	0
Consensus value	negative	positive	positive
Spiking	negative	positive	positive

##### Methods:

PCR = house method PCR

GS = Eurofins Genescan DNAanimal Ident

##### Comments:

The consensus values of the results of samples 1-3 are in qualitative agreement with the spiking of sample 2 (6,6% duck meat genus *anas*) and sample 3 (8,6% duck meat genus *anas*).

**Duck meat genus *anas*****Qualitative Valuation of Results**

Evaluation number	Sample 1	Sample 2	Sample 3	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
4	negative	positive	positive	3/3 (100%)	3/3 (100%)	ASU	
15	negative	positive	positive	3/3 (100%)	3/3 (100%)	GS	
11	negative	positive	positive	3/3 (100%)	3/3 (100%)	NGS	
2	negative	positive	positive	3/3 (100%)	3/3 (100%)	PCR	
14	negative	positive	positive	3/3 (100%)	3/3 (100%)	PCR	
19	negative	positive	positive	3/3 (100%)	3/3 (100%)	PCR	
20	negative	positive	positive	3/3 (100%)	3/3 (100%)	PCR	
22	negative	positive	positive	3/3 (100%)	3/3 (100%)	PCR / NGS	
6	negative	positive	positive	3/3 (100%)	3/3 (100%)	RF	
12	negative	positive	positive	3/3 (100%)	3/3 (100%)	RF	

	Sample 1	Sample 2	Sample 3
Number positive	0	10	10
Number negative	10	0	0
Percent positive	0	100	100
Percent negative	100	0	0
Consensus value	negative	positive	positive
Spiking	negative	positive	positive

**Methods:**

ASU = ASU §64 method

GS = Eurofins Genescan DNAanimal Ident

NGS = house method NGS

PCR = house method PCR

RF= RapidFinder™ ID Kit, ThermoFisher

**Comments:**

The consensus values of the results of samples 1-3 are in qualitative agreement with the spiking of sample 2 (6,6% duck meat genus *anas*) and sample 3 (8,6% duck meat genus *anas*).

**Duck meat genus *cairina*****Qualitative Valuation of Results**

Evaluation number	Sample 1	Sample 2	Sample 3	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
4	negative	negative	negative	3/3 (100%)	3/3 (100%)	ASU	
15	negative	negative	negative	3/3 (100%)	3/3 (100%)	GS	
11	negative	negative	negative	3/3 (100%)	3/3 (100%)	NGS	
14	negative	negative	negative	3/3 (100%)	3/3 (100%)	NGS	
19	negative	negative	negative	3/3 (100%)	3/3 (100%)	PCR	
22	negative	negative	negative	3/3 (100%)	3/3 (100%)	PCR / NGS	

	Sample 1	Sample 2	Sample 3
Number positive	0	0	0
Number negative	6	6	6
Percent positive	0	0	0
Percent negative	100	100	100
Consensus value	negative	negative	negative
Spiking	negative	negative	negative

**Methods:**

ASU = ASU §64 method

GS = Eurofins Genescan DNAanimal Ident

NGS = house method NGS

PCR = house method PCR

**Comments:**

The consensus values of the results of samples 1-3 are in qualitative agreement with the spiking of the samples. None of the samples were spiked with duck meat of the species *cairina*.

## 4.6 Proficiency Test Sheep meat

### 4.6.1 Immunological protein-based methods (e.g. ELISA, IEF, MALDI-TOF)

#### Sheep meat

#### Qualitative Valuation of Results

Evaluation number	Sample 1	Sample 2	Sample 3	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
6	positive	positive	positive	-	3/3 (100%)	ETM	

	Sample 1	Sample 2	Sample 3
Number positive	1	1	1
Number negative	0	0	0
Percent positive	100	100	100
Percent negative	0	0	0
Consensus value	-	-	-
Spiking	positive*	positive	positive

#### Methods:

ETM = ELISA-TEK™ Cooked Meat Species Kits

\* sample 1 was not spiked with sheep meat, but DNA from sheep was experimentally determined (see page 5)

#### Comments:

Only one participant submitted results obtained by protein-based methods (ELISA) for sheep meat. The results for samples 1-3 are in qualitative agreement with the spiking of sample 2 (43% sheep meat) and sample 3 (6,3% sheep meat), as well as the consensus values as determined by the DNA-based methods (see 4.6.2). Sample 1 was not spiked with meat from sheep, but sheep DNA was experimentally determined using PCR and NGS methods (see page 5 and 4.6.2).

#### 4.6.2 Molecular biological DNA-based methods (e.g. PCR, NGS, Microarray)

##### Sheep meat

##### Qualitative Valuation of Results

Evaluation number	Sample 1	Sample 2	Sample 3	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
4	positive	positive	positive	3/3 (100%)	3/3 (100%)	ASU	
9	positive	positive	positive	3/3 (100%)	3/3 (100%)	ASU	
17	positive	positive	positive	3/3 (100%)	3/3 (100%)	BP	
16	positive	positive	positive	3/3 (100%)	3/3 (100%)	FP	
23	positive	positive	positive	3/3 (100%)	3/3 (100%)	GI-S	
5	positive	positive	positive	3/3 (100%)	3/3 (100%)	GSD	
15	positive	positive	positive	3/3 (100%)	3/3 (100%)	GSD	
21	positive	positive	positive	3/3 (100%)	3/3 (100%)	ISO	
11a	positive	positive	positive	3/3 (100%)	3/3 (100%)	NGS	
1	positive	positive	positive	3/3 (100%)	3/3 (100%)	PCR	
2	positive	positive	positive	3/3 (100%)	3/3 (100%)	PCR	
3	positive	positive	positive	3/3 (100%)	3/3 (100%)	PCR	
7	negative*	positive	positive	2/3 (67%)	2/3 (67%)	PCR	
11b	positive	positive	positive	3/3 (100%)	3/3 (100%)	PCR	
13	positive	positive	positive	3/3 (100%)	3/3 (100%)	PCR	
14	positive	positive	positive	3/3 (100%)	3/3 (100%)	PCR	
18	positive	positive	positive	3/3 (100%)	3/3 (100%)	PCR	
19	positive	positive	positive	3/3 (100%)	3/3 (100%)	PCR	
20a	positive	positive	positive	3/3 (100%)	3/3 (100%)	PCR	
24	positive	positive	positive	3/3 (100%)	3/3 (100%)	PCR	
22	positive	positive	positive	3/3 (100%)	3/3 (100%)	PCR / NGS	
6	positive	positive	positive	3/3 (100%)	3/3 (100%)	RF	
12	positive	positive	positive	3/3 (100%)	3/3 (100%)	RF	
10	positive	positive	positive	3/3 (100%)	3/3 (100%)	SFA-4p	
20b	positive	positive	positive	3/3 (100%)	3/3 (100%)	SFA-4p	

	Sample 1	Sample 2	Sample 3
Number positive	24	25	25
Number negative	0	0	0
Percent positive	100	100	100
Percent negative	0	0	0
Consensus value	positive	positive	positive
Spiking	positive*	positive	positive

##### Methods:

ASU = ASU §64 method

BP = Bosphore Species Identification Kit, Anatolia Genew orks

FP = foodproof® Animal Detection LyoKit, Hygiena

GI-S = GEN-IAL First- Animal (single PCR)

GSD = DNAAnimal Ident, Gold Standard Diagnostics

ISO = ISO method

NGS = house method NGS

PCR = house method PCR

RF= RapidFinder™ ID Kit, ThermoFisher

SFA-4p = Sure Food Animal ID 4plex, R-Biopharm / Congen

\* sample 1 was not spiked with sheep meat, but DNA from sheep was experimental determined (see page 5)

##### Comments:

The consensus values of the results for samples 1-3 are in qualitative agreement with the spiking of sample 2 (43% sheep meat) and sample 3 (6,3% sheep meat). Sample 1 was not spiked with meat from sheep, but sheep DNA was experimental determined using Real Time PCR (see page 5).

## 5. Documentation

### 5.1 Details by the participants

#### 5.1.1 Immunological protein-based methods: Beef meat

Meth. Abbr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Limit of detection	Method
		Day/ Month	positive / negative	positive / negative	positive / negative	mg/kg	Test-Kit + Manufacturer
ETM	6	2025-04-30	positive	negative	positive	1	ETM = ELISA-TEK™ Cooked Meat Species Kits

Meth. Abbr.	Evaluation number	Method-No. / Test-Kit No.	Specificity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Antibody/Target-Sequence DNA	e.g. Extractionbuffer / Time / Temperature	
ETM	6				

#### 5.1.2 Immunological protein-based methods: Sheep meat

Meth. Abbr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Limit of detection	Method
		Day/ Month	positive / negative	positive / negative	positive / negative	mg/kg	Test-Kit + Manufacturer
ETM	6	2025-04-30	positive	positive	positive	1	ETM = ELISA-TEK™ Cooked Meat Species Kits

Meth. Abbr.	Evaluation number	Method-No. / Test-Kit No.	Specificity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Antibody/Target-Sequence DNA	e.g. Extractionbuffer / Time / Temperature	
ETM	6				



**5.1.3 DNA-based methods: Beef meat**

Meth. Abbr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Limit of detection	Method
		Day/ Month	positive / negative	positive / negative	positive / negative	mg/kg	Test-Kit + Manufacturer
ASU	9	2025-04-24	positive	negative	positive		ASU = ASU §64 Methode/method
ASU	4a	2025-04-11	positive	negative	positive	1	ASU = ASU §64 Methode/method
ASU	4b	2025-04-04	positive	negative	positive	0,1	ASU = ASU §64 Methode/method
BP	17	2025-03-28	positive	positive	positive		Bosphore Species Identification Kit
FP-1	16	2025-03-26	positive	positive	positive		RT-qPCR
GI-T	23	2025-04-17	positive	negative	positive		
GSD	5	2025-03-27	positive	negative	positive		GSD = DNAnimal Ident, Gold Standard Diagnostics
GSD	15	2025-04-02	positive	positive	positive		GSD = DNAnimal Ident, Gold Standard Diagnostics
ISO	1	2025-04-09	positive	negative	positive		div = house method PCR
NGS	11a	2025-04-28	positive	negative	positive		div = house method NGS
PCR	2	2025-03-25	positive	negative	positive		div = house method PCR
PCR	3	2025-05-12	positive	negative	positive	0,005%	div = house method PCR
PCR	8	2025-04-23	positive	negative	positive		div = house method PCR
PCR	13	2025-04-08	positive	negative	positive		div = house method PCR
PCR	14	2025-04-28	positive	positive	positive		div = house method PCR
PCR	18	2025-04-03	positive	positive	positive		Real Time PCR
PCR	19	2025-03-27	positive	negative	positive		div = house method PCR
PCR	24	2025-04-11	positive	negative	positive		
PCR	11b	2025-04-25	positive	negative	positive		div = house method PCR
PCR	20a	2025-04-08	positive	negative	positive	0,01	div = house method PCR
PCR / NGS	22	2025-04-22	positive	negative	positive		
RF	6	2025-05-01	positive	negative	positive		RF= RapidFinder™ ID Kit, ThermoFisher
RF	12	2025-04-29	positive	negative	positive		RF= RapidFinder™ ID Kit, ThermoFisher
SFA-4p	10	2025-03-26	positive	negative	positive		SFA-4p = Sure Food Animal ID 4plex, R-Biopharm / Congen
SFA-4p	20b	2025-04-08	positive	negative	positive	0,5	SFA-4p = Sure Food Animal ID 4plex, R-Biopharm / Congen

*continued next page*

## Continuation: DNA-based methods Beef meat

Meth. Abbr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Antibody /Target-Sequence DNA	e.g. Extractionbuffer / Time / Temperature	
ASU	9			Macherey & Nagel Nucleo Spin Food Kit	
ASU	4a	ASU L 00.00-184	16S rDNA	Extraction: Maxwell® RSC PureFood GMO and Authentication Kit (AS1600) Sequencing: Illumina MiSeq Database: MIDORI-Irna-Genbank257	
ASU	4b	ASU L 08.00-61	β-Actin	Extraction: Maxwell® RSC PureFood GMO and Authentication Kit (AS1600)	
BP	17			Magrev Tissue DNA Extraction Kit	GU
FP-1	16	foodproof® Animal Detection 1 LyoKit LP (Artikel-Nr. KIT230127), 2023-11	DNA	foodproof® Sample Preparation Kit III, Hygiena, (Artikel-Nr. KIT230174), Extraction Procedure C: Animal ID, Revision A, February 2024	In the kit named as bovine.
GI-T	23	GEN-IAL First Animal tetra PCR - Art.-No.: 10001300	DNA-Target	Extraction in 0.2 g & 2.0 g	
GSD	5				
GSD	15	GSD DNAnimal Multiplex			
ISO	1	ISO 20224-1-2020		Kingfisher MN Food	
NGS	11a				traces of cow DNA in sample 2 below LOD
PCR	2	Qiagen HT Kit Ref: 69571 Bovine Speciation Kit: 12019403	Bos Taurus	Extracted via Qiacube Automated extraction, Real Time PCR Detection with BioRad CFX96	CHC2399607, CHC2399608, CHC2399609
PCR	3	PG02 und PT01	DNA target Housekeeping Gen s PT01	höhere Einwaage, Chloroform bzw n-Heptan Reinigungsschritt zzgl und doppeltes CQW Wascschritt, AllMeat, AllHorse, AllMilch getestet Fa Microsynth	Nucleospin Foodkit optimiert und angewendet, bei Frischfleisch wird RNASE Schritt nach dem Poteinase K Verdau angewendet
PCR	8	BioRad, ID-Check Bovine Speciation Kit, Catalog#12019403		Congen, SureFood Prep Basic , S1052	
PCR	13				
PCR	14				
PCR	18				
PCR	19	PCR + QSEP		Maxwell® RSC PureFood GMO and Authentication Kit	
PCR	24	Real Time PCR			Liliana Vieira / Rita Melanda
PCR	11b				traces of cow DNA in sample 2 below LOD
PCR	20a	12019403	DNA-Target	iD Check Bio-Rad	
PCR / NGS	22			Real Time PCR and NGS methods	
RF	6	A24391			
RF	12	A24391 - 16224A082			
SFA-4p	10				
SFA-4p	20b	S6121	DNA-Target		

5.1.4 DNA-based methods: Goat meat

Meth. Abbr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Limit of detection	Method
		Day/ Month	positive / negative	positive / negative	positive / negative	mg/kg	Test-Kit + Manufacturer
ASU	4	2025-04-11	positive	negative	positive	1	ASU = ASU §64 Methode/method
ASU	9	2025-04-24	positive	positive	positive		ASU = ASU §64 Methode/method
BP	17	2025-03-28	positive	negative	positive		Bosphore Species Identification Kit
GI-S	23	2025-04-17	positive	positive	positive		GI-S = GEN-IAL First- Animal (single PCR)
GS	15	2025-04-02	positive	negative	positive		GS = Eurofins Genescan DNAnimal Ident
GSD	5	2025-04-09	positive	negative	positive		GSD = DNAnimal Ident, Gold Standard Diagnostics
ISO	21	2025-04-10	positive	negative	positive		ISO/TS 20224
NGS	11a	2025-04-28	positive	negative	positive		div = house method NGS
PCR	1	2025-04-02	positive	negative	positive	1	div = house method PCR
PCR	2	2025-03-25	positive	negative	positive		div = house method PCR
PCR	3	2025-05-12	positive	positive	positive	0,005%	div = house method PCR
PCR	11b	2025-04-25	positive	negative	positive		div = house method PCR
PCR	13	2025-04-08	positive	negative	positive		div = house method PCR
PCR	14	2025-04-28	positive	negative	positive		div = house method PCR
PCR	16	2025-03-26	positive	positive	positive		RT-qPCR
PCR	18	2025-04-03	positive	negative	positive		Real Time PCR
PCR	19	2025-03-27	positive	negative	positive		div = house method PCR
PCR	20a	2025-04-08	positive	negative	positive	0,01	div = house method PCR
PCR	24	2025-04-11	positive	negative	positive		
PCR / NGS	22	2025-04-22	positive	negative	positive		
RF	6	2025-05-01	positive	negative	positive		RF= RapidFinder™ ID Kit, ThermoFisher
RF	12	2025-04-29	positive	negative	positive		RF= RapidFinder™ ID Kit, ThermoFisher
SFA-4p	10	2025-03-26	positive	negative			SFA-4p = Sure Food Animal ID 4plex, R-Biopharm / Congen
SFA-4p	20b	2025-04-08	positive	negative	positive	0,5	SFA-4p = Sure Food Animal ID 4plex, R-Biopharm / Congen

continued next page

## Continuation: DNA-based methods Goat meat

Meth. Abbr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Antibody /Target-Sequence DNA	e.g. Extractionbuffer / Time / Temperature	
ASU	4	ASU L 00.00-184	16S rDNA	Extraction: Maxwell® RSC PureFood GMO and Authentication Kit (AS1600) Sequencing: Illumina MiSeq Database: MIDORI-Irna-Genbank257	
ASU	9			Macherey & Nagel Nucleo Spin Food Kit	
BP	17			Magrev Tissue DNA Extraction Kit	GU
GI-S	23	Art.-No.: 10001247	DNA-Target	Extraction in 0.2 g & 2.0 g	
GS	15	GSD DNAnimal Multiplex			
GSD	5				
ISO	21	ISO/TS 20224-5:2020; TCVN 13842-5:2023			
NGS	11a				traces of goat DNA in sample 2 below LOD
PCR	1	ISO 20224-5-2020		Kingfisher MN Food	
PCR	2	Qiagen HT Kit Ref: 69571 Goat Speciation Kit: 12019404	Capra Hircus	Extracted via Qiacube Automated extraction, Real Time PCR Detection with BioRad CFX96	CHC2399607, CHC2399608, CHC2399609
PCR	3	PG02 und PT01	DNA target Housekeeping Gen s PT01	höhere Einwaage, Chloroform bzw n-Heptan Reinigungsschritt zzgl und doppeltes CQW Waschschrift, AllMeat, AllHorse, AllMilch getestet Fa Microsynth	Nucleospin Foodkit optimiert und angewendet, bei Frischfleisch wird RNASE Schritt nach dem Poteinase K Verdau angewendet
PCR	11b				traces of goat DNA in sample 2 below LOD
PCR	13				
PCR	14				
PCR	16	foodproof® Goat Species Detection Kit (Artikel-Nr. KIT 230227), 2023-12	DNA	foodproof® Sample Preparation Kit III, Hygiena, (Artikel-Nr. KIT230174), Extraction Procedure C: Animal ID, Revision A, February 2024	
PCR	18				
PCR	19	PCR + QSEP		Maxwell® RSC PureFood GMO and Authentication Kit	
PCR	20a	12019404	DNA-Target	iD Check Bio-Rad	
PCR	24	Real Time PCR			Liliana Vieira / Rita Melanda
PCR / NGS	22			Real Time PCR and NGS methods	
RF	6	A24407			
RF	12	IMG320 - 32024A017			
SFA-4p	10				
SFA-4p	20b	S6121	DNA-Target		

5.1.5 DNA-based methods: Poultry meat in general

Meth. Abbr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Limit of detection	Method
		Day/ Month	positive / negative	positive / negative	positive / negative	mg/kg	Test-Kit + Manufacturer
FP-2	16	2025-03-26	positive	positive	positive		RT-qPCR
GI-T	23	2025-04-17	negative	negative	negative		
NGS	11a	2025-04-28	positive	positive	positive		div = house method NGS
PCR	1	2025-04-29	positive	positive	positive		div = house method PCR
PCR	18	2025-04-10	positive	positive	negative		Real Time PCR
PCR	11b	2025-04-25	positive	positive	positive		div = house method PCR
PCR / NGS	22	2025-04-22	positive	positive	negative		
RF	6	2025-05-01	positive	positive	positive		RF= RapidFinder™ ID Kit, ThermoFisher
SFA-4p	8	2025-04-23	positive	positive			SFA-4p = Sure Food Animal ID 4plex, R-Biopharm / Congen

Meth. Abbr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Antibody / Target-Sequence DNA	e.g. Extractionbuffer / Time / Temperature	
FP-2	16	foodproof® Animal Detection 2 LyoKit (Artikel-Nr. KIT230032), 2023-11	DNA	foodproof® Sample Preparation Kit III, Hygiena, (Artikel-Nr. KIT230174), Extraction Procedure C: Animal ID, Revision A, February 2024	The kit detects: chicken (Gallus gallus), turkey (Meleagris gallopavo) and poultry/aves (includes Anser, Anas, Struthio, Gallus, Meleagris, Numida & other birds. Poultry is able to be detected with this kit so far.
GI-T	23	GEN-IAL First Animal tetra PCR - Art.-No.: 10001300	DNA-Target	Extraction in 0.2 g & 2.0 g	
NGS	11a				
PCR	1			Kingfisher MN Food	The result is given as presence of chicken and duck
PCR	18				
PCR	11b				
PCR / NGS	22			Real Time PCR and NGS methods	Detection of poultry (chicken and turkey) DNA using real-time PCR
RF	6	A24397			
SFA-4p	8	S6123, Pork, Chicken, Turkey		Congen, SureFood Prep Basic , S1052	

5.1.6 DNA-based methods: Chicken meat

Meth. Abbr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Limit of detection	Method
		Day/ Month	positive / negative	positive / negative	positive / negative	mg/kg	Test-Kit + Manufacturer
ASU	4a	2025-04-11	positive	positive	negative	1	ASU = ASU §64 Methode/method
ASU	4b	2025-04-04	positive	positive	negative	0,1	ASU = ASU §64 Methode/method
ASU	9	2025-04-24	positive	positive	negative		ASU = ASU §64 Methode/method
BP	17	2025-03-28	positive	positive	negative		Bosphore Species Identification Kit
FP-2	16	2025-03-26	positive	positive	negative		RT-qPCR
GI-T	23	2025-04-17	positive	positive	negative		
GS	5	2025-03-27	positive	positive	negative		GS = Eurofins Genescan DNAAnimal Ident
GS	15	2025-04-02	positive	positive	negative		GS = Eurofins Genescan DNAAnimal Ident
ISO	21	2025-04-10	positive	positive	negative		ISO/TS 20224
NGS	11a	2025-04-28	positive	positive	negative		div = house method NGS
PCR	1	2025-04-09	positive	positive	negative		div = house method PCR
PCR	2	2025-03-25	positive	positive	negative		div = house method PCR
PCR	3	2025-05-12	positive	positive	negative	0,005%	div = house method PCR
PCR	7	2025-04-17	positive	negative	negative		div = house method PCR
PCR	11b	2025-04-25	positive	positive	negative		div = house method PCR
PCR	13	2025-04-08	positive	positive	negative		div = house method PCR
PCR	14	2025-04-28	positive	positive	negative		div = house method PCR
PCR	18	2025-04-10	positive	positive	negative		Real Time PCR
PCR	19	2025-03-27	positive	positive	negative		div = house method PCR
PCR	20a	2025-04-08	positive	positive	negative	0,01	div = house method PCR
PCR	24	2025-04-11	positive	positive	negative		
PCR / NGS	22	2025-04-22	positive	positive	negative		
RF	6	2025-05-01	positive	positive	negative		RF= RapidFinder™ ID Kit, ThermoFisher
RF	12	2025-04-29	positive	positive	negative		RF= RapidFinder™ ID Kit, ThermoFisher
SFA-4p	8	2025-04-23	positive	positive	negative		SFA-4p = Sure Food Animal ID 4plex, R-Biopharm / Congen
SFA-ID	20b	2025-04-08	positive	positive	negative	0,5	SFA-ID = Sure Food Animal ID, R-Biopharm / Congen

continued next page

## Continuation: DNA-based methods Chicken meat

Meth. Abbr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Antibody/Target-Sequence DNA	e.g. Extractionbuffer / Time / Temperature	
ASU	4a	ASU L 00.00-184	16S rDNA	Extraction: Maxwell® RSC PureFood GMO and Authentication Kit (AS1600) Sequencing: Illumina MiSeq Database: MIDORI-Irna-Genbank257	
ASU	4b	ASU L 08.00-61	TF-GB3	Extraction: Maxwell® RSC PureFood GMO and Authentication Kit (AS1600)	
ASU	9			Macherey & Nagel Nucleo Spin Food Kit	
BP	17			Magrev Tissue DNA Extraction Kit	GU
FP-2	16	foodproof® Animal Detection 2 LyoKit (Artikel-Nr. KIT230032), 2023-11	DNA	foodproof® Sample Preparation Kit III, Hygiena, (Artikel-Nr. KIT230174), Extraction Procedure C: Animal ID, Revision A, February 2024	
GI-T	23	GEN-IAL First Animal tetra PCR - Art.-No.: 10001300	DNA-Target	Extraction in 0.2 g & 2.0 g	
GS	5				
GS	15	GSD DNAnimal Multiplex			
ISO	21	ISO/TS 20224-4:2020; TCVN 13842-4:2023			
NGS	11a				traces of chicken DNA in sample 3 below LOD
PCR	1	ISO 20224-4-2020		Kingfisher MN Food	
PCR	2	Qiagen HT Kit Ref: 69571 Chicken Speciation Kit: 12019421	Gallus Gallus	Extracted via Qiacube Automated extraction, Real Time PCR Detection with BioRad CFX96	CHC2399607, CHC2399608, CHC2399609
PCR	3	PG02 und PT01	DNA target Housekeeping Gen s PT01	höhere Einwaage, Chloroform bzw n-Heptan Reinigungsschritt zzgl und doppeltes CQW Wascschritt, AllMeat, AllHorse, AllMilch getestet Fa Microsynth	Nucleospin Foodkit optimiert und angewendet, bei Frischfleisch wird RNASE Schritt nach dem Poteinase K Verdau angewendet
PCR	7			Extraction and amplification	
PCR	11b				
PCR	13				
PCR	14				
PCR	18				
PCR	19	PCR + QSEP		Maxwell® RSC PureFood GMO and Authentication Kit	
PCR	20a	12019421	DNA-Target	iD Check Bio-Rad	
PCR	24	Real Time PCR			Liliana Vieira / Rita Melanda
PCR / NGS	22			Real Time PCR and NGS methods	
RF	6	A24393			
RF	12	A24393 - 16825A067			
SFA-4p	8	S6123, Pork, Chicken, Turkey		Congen, SureFood Prep Basic , S1052	
SFA-ID	20b	S6115	DNA-Target		

5.1.7 DNA-based methods: Duck meat (genus anas)

Meth. Abbr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Limit of detection	Method
		Day/ Month	positive / negative	positive / negative	positive / negative	mg/kg	Test-Kit + Manufacturer
ASU	4	2025-04-11	negative	positive	positive	1	ASU = ASU §64 Methode/method
GS	5	2025-03-26	negative	positive	positive		GS = Eurofins Genescan DNAnimal Ident
GS	15	2025-04-02	negative	positive	positive		GS = Eurofins Genescan DNAnimal Ident
NGS	11	2025-04-28	negative	positive	positive		div = house method NGS
PCR	1	2025-03-27	negative				div = house method PCR
PCR	2	2025-03-25	negative	positive	positive		div = house method PCR
PCR	13	2025-04-08	negative	positive	positive		div = house method PCR
PCR	14	2025-04-28	negative	positive	positive		div = house method PCR
PCR	16	2025-03-26	positive	positive	positive		RT-qPCR
PCR	19	2025-03-27	negative	positive	positive		div = house method PCR
PCR	20	2025-04-08	negative	positive	positive	0,01	div = house method PCR
PCR / NGS	22	2025-04-22	negative	positive	positive		
RF	6	2025-05-01	negative	positive	positive		RF= RapidFinder™ ID Kit, ThermoFisher
RF	12	2025-04-29	negative	positive	positive		RF= RapidFinder™ ID Kit, ThermoFisher

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## Continuation: DNA-based methods Duck meat (genus anas)

Meth. Abbr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Antibody/Target-Sequence DNA	e.g. Extractionbuffer / Time / Temperature	
ASU	4	ASU L 00.00-184	16S rDNA	Extraction: Maxwell® RSC PureFood GMO and Authentication Kit (AS1600) Sequencing: Illumina MiSeq Database: MIDORI-Irna-Genbank257	
GS	5				we cannot distinguish between Genus anas and cairina, so Or sample 2 and 3 we cannot say if both genus are positive or only one
GS	15	GSD DNAnimal Multiplex			
NGS	11				
PCR	1		the method does not descriminate between anas and cairina	Kingfisher MN Food	samples 2 and 3 were positive for duck.
PCR	2	Qiagen HT Kit Ref: 69571 Duck Speciation Kit: 12019411	Anas Platyrhynchos	Extracted via Qiacube Automated extraction, Real Time PCR Detection with BioRad CFX96	CHC2399607, CHC2399608, CHC2399609
PCR	13				zwischen den beiden Enten-Arten kann mit der verwendeten Methode nicht unterschieden werden.
PCR	14				
PCR	16	foodproof® Animal Detection 2 LyoKit (Artikel-Nr. KIT230032), 2023-11	DNA	foodproof® Sample Preparation Kit III, Hygiena, (Artikel-Nr. KIT230174), Extraction Procedure C: Animal ID, Revision A, February 2024	The kit detects: chicken (Gallus gallus), turkey (Meleagris gallopavo) and poultry/aves (includes Anser, Anas, Struthio, Gallus, Meleagris, Numida & other birds. The genus Anas could be detected with this kit so far.
PCR	19	PCR + QSEP		Maxwell® RSC PureFood GMO and Authentication Kit	
PCR	20	12019411	DNA-Target	iD Check Bio-Rad	
PCR / NGS	22			Real Time PCR and NGS methods	
RF	6	IMG - 241			
RF	12	IMG241 - 35024A009		Kit labelled "Imagen" not "RapidFinder", but still ThermoFisher.	

5.1.8 DNA-based methods: Duck meat (genus *cairina*)

Meth. Abbr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Limit of detection	Method
		Day/ Month	positive / negative	positive / negative	positive / negative	mg/kg	Test-Kit + Manufacturer
ASU	4	2025-04-11	negative	negative	negative	1	ASU = ASU §64 Methode/method
GS	5	2025-03-26	negative	positive	positive		GS = Eurofins Genescan DNAnimal Ident
GS	15	2025-04-02	negative	negative	negative		GS = Eurofins Genescan DNAnimal Ident
NGS	11	2025-04-28	negative	negative	negative		div = house method NGS
NGS	14	2025-04-28	negative	negative	negative	1	div = house method NGS
PCR	1	2025-03-27	negative				div = house method PCR
PCR	13	2025-04-08	negative	positive	positive		div = house method PCR
PCR	19	2025-03-27	negative	negative	negative		div = house method PCR
PCR / NGS	22	2025-04-22	negative	negative	negative		

Meth. Abbr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Antibody /Target-Sequence DNA	e.g. Extractionbuffer / Time / Temperature	
ASU	4	ASU L 00.00-184	16S rDNA	Extraction: Maxwell® RSC PureFood GMO and Authentication Kit (AS1600) Sequencing: Illumina MiSeq Database: MIDORI-Irna-Genbank257	
GS	5				we cannot distinguish between Genus anas and cairina, so Or sample 2 and 3 we cannot say if both genus are positive or only one
GS	15	GSD DNAnimal Multiplex			
NGS	11				
NGS	14				
PCR	1		the method does not discriminate between anas and cairina	Kingfisher MN Food	samples 2 and 3 were positive for duck.
PCR	13				zwischen den beiden Enten-Arten kann mit der verwendeten Methode nicht unterschieden werden.
PCR	19	PCR + QSEP		Maxwell® RSC PureFood GMO and Authentication Kit	
PCR / NGS	22			Real Time PCR and NGS methods	

**5.1.9 DNA-based methods: Sheep meat**

Meth. Abbr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Limit of detection	Method
		Day/ Month	positive / negative	positive / negative	positive / negative	mg/kg	Test-Kit + Manufacturer
ASU	4	2025-04-11	positive	positive	positive	1	ASU = ASU §64 Methode/method
ASU	9	2025-03-24	positive	positive	positive		ASU = ASU §64 Methode/method
BP	17	2025-03-28	positive	positive	positive		Bosphore Species Identification Kit
FP	16	2025-03-26	positive	positive	positive		RT-qPCR
GI-S	23	2025-04-17	positive	positive	positive		GI-S = GEN-IAL First- Animal (single PCR)
GSD	5	2025-03-28	positive	positive	positive		GSD = DNAnimal Ident, Gold Standard Diagnostics
GSD	15	2025-04-02	positive	positive	positive		GSD = DNAnimal Ident, Gold Standard Diagnostics
ISO	21	2025-04-10	positive	positive	positive		ISO/TS 20224
NGS	11a	2025-04-28	positive	positive	positive		div = house method NGS
PCR	1	2025-04-07	positive	positive	positive		div = house method PCR
PCR	2	2025-03-25	positive	positive	positive		div = house method PCR
PCR	3	2025-05-12	positive	positive	positive	0,005%	div = house method PCR
PCR	7	2025-04-10	negative	positive	positive		div = house method PCR
PCR	11b	2025-04-25	positive	positive	positive		div = house method PCR
PCR	13	2025-04-08	positive	positive	positive		div = house method PCR
PCR	14	2025-04-28	positive	positive	positive		div = house method PCR
PCR	18	2025-04-03	positive	positive	positive		Real Time PCR
PCR	19	2025-03-27	positive	positive	positive		div = house method PCR
PCR	20a	2025-04-08	positive	positive	positive	0,01	div = house method PCR
PCR	24	2025-04-16	positive	positive	positive		div = house method PCR
PCR / NGS	22	2025-04-22	positive	positive	positive		
RF	6	2025-05-01	positive	positive	positive		RF= RapidFinder™ ID Kit, ThermoFisher
RF	12	2025-04-29	positive	positive	positive		RF= RapidFinder™ ID Kit, ThermoFisher
SFA-4p	10	2025-03-26	positive	positive	positive		SFA-4p = Sure Food Animal ID 4plex, R-Biopharm / Congen
SFA-4p	20b	2025-04-08	positive	positive	positive	0,5	SFA-4p = Sure Food Animal ID 4plex, R-Biopharm / Congen

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## Continuation: DNA-based methods Sheep meat

Meth. Abbr.	Evaluation number	Method-No. / Test-Kit No.	Specificity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Antibody/Target-Sequence DNA	e.g. Extractionbuffer / Time / Temperature	
ASU	4	ASU L 00.00-184	16S rDNA	Extraction: Maxwell® RSC PureFood GMO and Authentication Kit (AS1600) Sequencing: Illumina MiSeq Database: MIDORI-Irna-Genbank257	
ASU	9			Macherey & Nagel Nucleo Spin Food Kit	
BP	17			Magrev Tissue DNA Extraction Kit	GU
FP	16	foodproof(R) SL Sheep Species Detection Kit, Hygiena, Product Number: KIT230224, 2023-12	DNA	foodproof® Sample Preparation Kit III, Hygiena, (Artikel-Nr. KIT230174), Extraction Procedure C: Animal ID, Revision A, February 2024	
GI-S	23	Art.-No.: 10001248	DNA-Target	Extraction in 0.2 g & 2.0 g	
GSD	5				
GSD	15	GSD DNA Animal Multiplex			
ISO	21	ISO/TS 20224-2:2020; TCVN 13842-2:2023			
NGS	11a				
PCR	1	Publication		Kingfisher MN Food	
PCR	2	Qiagen HT Kit Ref: 69571 Sheep Speciation Kit: 12019393	Ovis Aries	Extracted via Qiacube Automated extraction, Real Time PCR Detection with BioRad CFX96	CHC2399607, CHC2399608, CHC2399609
PCR	3	PG02 und PT01	DNA target Housekeeping Gen s PT01	höhere Einwaage, Chloroform bzw n-Heptan Reinigungsschritt zzgl und doppeltes CQW Wascschritt, AllMeat, AllHorse, AllMilch getestet Fa Microsynth	Nucleospin Foodkit optimiert und angewendet, bei Frischfleisch wird RNASE Schritt nach dem Poteinase K Verdau angewendet
PCR	7			Extraction and amplification	
PCR	11b				
PCR	13				
PCR	14				
PCR	18				
PCR	19	PCR + QSEP		Maxwell® RSC PureFood GMO and Authentication Kit	
PCR	20a	12019393	DNA-Target	iD Check Bio-Rad	
PCR	24	Real Time PCR			Liliana Vieira / Rita Melanda
PCR / NGS	22			Real Time PCR and NGS methods	
RF	6	A24395			
RF	12	A24395 - 17124A059			
SFA-4p	10				
SFA-4p	20b	S6121	DNA-Target		

## 5.2 Information on the Proficiency Test (PT)

Before the PT, the participants received the following information in the sample cover letter:

PT number	<b>DLA ptAUS1 (2025)</b>
PT name	<b>Animal Species-Screening I – 3 Samples qualitative: Beef, Goat, Poultry (Chicken and Duck (genus: anas or cairina)) and Sheep in cooked Meat Product</b>
Sample matrix*	Samples 1-3: Sausage Meat (heated)/ ingredients: various meat species, water, gelatine (pork), salt and sorbic acid
Number of samples and sample amount	3 different Samples 1-3: 30 g each
Storage	Samples 1-3: cooled 2 - 10°C (long term frozen < -18°C)
Intentional use	Laboratory use only (quality control samples)
Parameter	Qualitative: Beef, Goat, Poultry (Chicken and Duck) and Sheep Samples 1-3: appr. 1-90%
Methods of analysis	The analytical methods are optional
Notes to analysis	The analysis of PT samples should be performed like a routine laboratory analysis. In general we recommend to homogenize a representative sample amount before analysis according to good laboratory practice, especially in case of low sample weights.
Result table	One result each should be determined for Samples 1-3. The results should be filled in the result entry table.
Units	positive / negative (limit of detection %)
Number of significant digits	at least 2
Further information	Further information can be given in the result submission file.
Result submission	online via <b>my DLA   participant's portal</b> ( <a href="https://my.dla-pt.com">https://my.dla-pt.com</a> ) you will receive further information about the access by e-mail
Last Deadline	<b>the latest May 2025-05-02</b>
Evaluation report	The evaluation report is expected to be completed 6 weeks after deadline of result submission and sent as PDF file by e-mail.
Coordinator and contact person of PT	Alexandra Scharf PhD

\* Control of mixture homogeneity and qualitative testings are carried out by DLA. Further testing of the content, homogeneity and stability of PT parameters can be subcontracted by DLA.

## 6. Index of participant laboratories in alphabetical order

Participant	Town	Country
		GB
		CH
		ES
		MY
		DE
		CY
		IT
		IT
		FR
		DE
		GB
		DE
		PT
		TR
		VN
		DE
		DE
		AT
		GB
		DE
		CH
		GR
		GB
		DE

*[The address data of the participants were deleted for publication of the evaluation report.]*

## 7. Index of references

The list of **references no. 1-21** can be found in the “**DLA Evaluation Guide 02.02 (2024) General Proficiency Test Schemes**”.

***Additional specific references:***

- A) Lebensmittelchemische Gesellschaft [LChG der GDCh] "Statement of the WG on: Methods for the Differentiation of Animal Species in Food - Status quo, (2016), Food Chemistry Society of the GDCh.