ANNEX 1.1B REGULATIONS FOR SAMPLING HYGIENOGRAM

A. introduction

A hygienogram provides a picture of the hygiene status of the barn sampled. A number of comments are needed with regard to this sampling method (for example, the fact that not all plates are fully sampled, such as the difference in contact surface between a barn floor and a slat). Nevertheless, the general view is that this method provides a qualitatively acceptable picture of the hygiene status, allowing action to be taken.

This annex describes the sites where samples must be taken, the method to be used (impression) and the method of dispatching the samples to the HOSOWO authority authorised to analyse (incubate and count) the samples and write up the result. The requirements for carrying out these analyses can be found in Annex 1.1C. The sections in italics in this annex serve as guidance for the HOSOWO participant.

As mentioned, the sites that must be sampled are described in this annex. The regulations pertaining to this are subdivided into three types of barn:

- Floor barn (broiler barn, turkey barns etc. no slats);
- Aviary and free-range barns;
- Cage/colony housing incl. patio barns.

Samples for a hygienogram are taken in barns that have recently been cleaned and disinfected. Make sure that it is safe to enter the barn; there may be some disinfectant residues still present. As a guideline, the barn was ventilated for at least two hours before sampling and the barn was cleaned and/or disinfected the day before sampling.

To create a hygienogram, RODAC plates filled with agar of a specific composition are used. Four compositions are permitted. Where the stated compositions are deviated from, the authority must demonstrate that they are at least equivalent to equal results.

Composition 1
(in grams per litre, unless stated otherwise):

Nutrient Broth no. 2	25
Agar	16
Sodium thiosulphate	0.5
Tween 80	1 ml
Ammonium carbonate	1
Lecithin	2
L-histidine	1

Composition 2
(in grams per litre, unless stated otherwise):

Agar	18
Tween 80	5 ml
Lecithin	0.7
Tryptone	15
Soya peptone	5
Sodium chloride	5
Histidine	1

Composition 3
(in grams per litre, unless stated otherwise):

Agar	16
Tween 80	1 ml
Ammonium carbonate	1
Lecithin	2
L-histidine	1
Sodium chloride	5
Meat extract	10
Peptone (tryptone + meat peptone)	10
Sodium thiosulphate (5H2O)	0.5

Composition 4
(in grams, unless stated otherwise):

Agar	18
Tween 80	5 ml
Lecithin	0.7
Histidine	1
Soymeal peptone	5
Casein peptone	15
NaCl	5

The sites to be sampled are identified with a code (letter or number). The codes are based on the following (hypothetical) barn layout:

	A	B	C	D	E	F
1						
2						
3						

The barn is divided into six strips lengthways (A-F) and three strips widthways (1-3), making 18 sections altogether.

B. <u>Method</u>

1. RODAC plates with a diameter of 5.5 cm are used.
2. The production date of the RODAC plates is stated on every pack.
3. The expiry date is stated on every pack.
4. RODAC plates are stored lid side down.
5. Sampler wears farm-owned clothing and footwear. If not present, or not sufficiently clean, disposable clothing and/or overshoes are also permitted.
6. The appropriate hygienogram form is completed (see specimen form in Annex 1.1C).
   It is permissible to use a different form as long as all the information and comments as requested are stated.
7. Sampling is carried out in accordance with the appropriate sampling scheme.
   Wet sites in the barn are not sampled.
8. The RODAC plate containing the agar is pressed down onto the surface to be tested for 5 seconds. The agar does not come into contact with the hands.
9. The RODAC plate must not be moved while the impression is being made (if it is not practical to avoid movement, keep movement to a minimum).
10. The lid is replaced after the impression is made.
11. The RODAC plate is placed lid side down in a protective cover.
12. No RODAC plates are used:
    a. if condensation has occurred on the inside of the plates;
    b. if the plates have been opened without taking an impression;
    c. if growth can be seen on the agar;
    d. if the expiry date on the plates has expired;
    e. if the plate is cracked or broken.
13. Samples are taken as follows:

- **<u>Floor barn</u>**: The barn is divided into six equal parts lengthways (A-F) and three sections widthways (1-3). See the introduction for more information on the barn layout. Samples can be coded differently from the numbers and letters mentioned above, provided the coding used is unique and traceable. Samples are then taken in the following sections:
  a. A - F: floor (6 plates) (nos. 1.1 - 1.6)

b. A, BC, DE and F: feeding system (= 4 plates) (nos. 2.1 -2.4)
Preferably on the inside (of the pan or trough).
c. A and F: drinking system (=2 plates) (nos. 3.1 - 3.2)
d. 1, 2, 3, A and F: wall (= 5 plates) (nos. 4.1 -4.5)
From the top end(s), between 1 m and 2 m high.
e. 1 and 3: ceiling (= 2 plates) (nos 5.1 and 5.2)
If inaccessible, take impressions as high up on the wall as possible
f. 1 and 3: inlets/wall of inlet inside (= 2 plates) (nos 6.1 and 6.2)
Sampling is carried out on the side wall of the valve opening on the barn side of the valve. With an inlet, sampling is done on the side of the inlet.
If inaccessible, take impressions as close as possible to the inlet.
g. Inside the surface of one random feed hopper (= 1 plate) (no. 7.1)
If the inside of the feed hopper is inaccessible, take an impression from the outside of the feed hopper.

- **Aviary and free-range barns**: The barn is divided into six equal parts lengthways (A-F) and three sections widthways (1-3). See the introduction for more information on the barn layout. Samples can be coded differently from the numbers and letters mentioned above, provided the coding used is unique and traceable. Samples are then taken in the following sections:
  a. A - F: floor (6 plates) (nos. 1.1 - 1.6)
  b. AB, CD and EF: feeding system (= 3 plates) (nos. 2.1 to 2.3)
  Preferably on the inside (of the pan or trough).
  c. AB and EF: drinking system (=2 plates) (nos. 3.1 - 3.2)
  d. 1, 2, 3 and 3: wall (= 3 plates) (nos. 4.1 -4.3)
  From one of the two top ends between 1 m and 2 m high.
  e. 1 and 3: ceiling (= 2 plates) (nos 5.1 and 5.2)
  If inaccessible, take impressions as high up on the wall as possible.
  f. 1 and 3: inlets/wall of the inlet inside (= 2 plates) (nos 6.1 and 6.2)
  Sampling is carried out on the side wall of the valve opening on the barn side of the valve. With an inlet, sampling is done on the side of the inlet.
  If inaccessible, take impressions as close as possible to the inlet.
  g. Inside surface of one random feed hopper (= 1 plate) (no. 7.1)
  If the inside of the feed hopper is inaccessible, take an impression from the outside of the feed hopper.
  h. <u>If present:</u> ABC and DEF: laying nest (various system rows) (= 2 plates) (nos. 8.1 - 8.2)
  i. <u>If present:</u> 1 and 3: egg belt = (2 plates) (nos. 9.1 and 9.2)
  j. <u>If present:</u> ABC and DEF: horizontal mesh (= 2 plates) (nos. 10.1 and 10.2)

- **Cage/colony housing**: The barn is divided into six equal parts lengthways (A-F) and three sections widthways (1-3). See the introduction for more information on the barn layout. Samples can be coded differently from the numbers and letters mentioned above, provided the coding used is unique and traceable. Samples are then taken in the following sections:
    a. AB, CD and EF: path between cages (=3 plates) (nos. 1.1 - 1.3)
    b. AB, CD and EF: feeding system (= 3 plates) (nos. 2.1 to 2.3)
    Preferably on the inside (of the pan or trough).
    c. AB and EF: drinking system (=2 plates) (nos. 3.1 - 3.2)
    d. 2 and 3: cage wall (= 2 plates) (nos. 4.1 - 4.2)
    e. A and F: wall (=2 plates) (nos. 5.1 and 5.2)
    From the barn wall, between 1 and 2 m high
    f. 1 and 3: ceiling (= 2 plates) (nos 6.1 and 6.2)
    If inaccessible, take impressions as high up on the wall as possible.
    g. 1 and 3: valves/wall of the inlet inside (= 2 plates) (nos 7.1 and 7.2)
    Sampling is carried out on the side wall of the valve opening on the barn side of the valve. With an inlet, sampling is done on the side of the inlet.
    If inaccessible, take impressions as close as possible to the inlet.
    h. Inside surface of one random feed hopper (= 1 plate) (no. 8.1)
    If the inside of the feed hopper is inaccessible, take an impression from the outside of the feed hopper.
    i. ABC and DEF: cage floor (= 2 plates) (nos. 9.1 - 9.2)
    j. If present: ABC and DEF: laying nest (various system rows) (= 2 plates) (nos. 10.1 - 10.2)
    k. If present: 1 and 3: egg belt = (2 plates) (nos. 11.1 and 11.2)

14. In addition to the samples taken in accordance with the sampling scheme, the following two samples are taken:
    a. One RODAC plate is not sampled.
    Negative sample: as a control for the plates used.
    b. One RODAC plate is sampled inside the farm gate, but outside the barn.
    Positive sample: as a control for the plates used.
15. The method of handling of sampled RODAC plates is intended to prevent contamination or cross-contamination.
16. The transport of the sampled RODAC plates takes place at a temperature between 0˚C and 25˚C, avoiding exposure to sunlight and excessive heat.
    The authority can demonstrate this by placing a min-max thermometer and logbook. The maximum temperature is noted in the logbook.
17. RODAC plates must be sent within 24 hours after sampling.
    This must not be left to the poultry farmer/client or other similar party.
18. The RODAC plates must be sent, stating the information referred to in Annex 1.1C, to a HOSOWO authority that is approved to analyse hygienograms.
    This can be the same authority as the sampling authority. The authority can demonstrate this with a shipping note, invoice or similar.

ANNEX 1.1C REGULATIONS FOR ANALYSIS HYGIENOGRAM

A. introduction

A hygienogram provides a picture of the hygiene status of the barn sampled. Annex 1.1B describes how the approved HOSOWO authority should take the samples. The method of analysis (incubating and counting) and the form for the results are described in this annex. The results must be reported on the relevant hygienogram form (or a similar document). The forms concerned can be found at the end of this annex.

B. **Method**

STANDARD	REGULATION	INTERPRETATION OF THE REGULATION
C01	Sampled RODAC plates are stored at a temperature of between 0º and 20ºC.	The authority can demonstrate this (e.g. by placing a thermometer and logbook).
C02	Incubation should ideally start on the day of receipt, but within 24 hours after receipt at the latest.	It is not permitted to leave sampled plates unincubated for more than one day (after receipt).
C03	The RODAC plates are incubated at 37ºC for 18- 24 hours. The temperature of the incubator is measured when the samples are placed into the incubator and when the samples are removed from the incubator using a calibrated thermometer, display or logger.	A deviation of +/- 1ºC is permissible. The authority can demonstrate this, for example with a logbook.
C04	The thermometer(s) is/are calibrated every year.	At least one calibrated thermometer is present, with a calibration report. The other thermometers, displays or loggers are inspected for accuracy annually by the HOSOWO authority using this calibrated thermometer.
C05	The RODAC plates are read immediately after incubation or are stored in cooled conditions after incubation for max. 48 hours (2˚C-8˚C) and then read.	
C06	The number of colony-forming units (CFU) is counted.	
C07	If the plate becomes overgrown with one single spreader, this is regarded as 1 CFU.	A spreading bacterium spreads its growth over the plate, so that the bacteria underneath can no longer be assessed.

STANDARD	REGULATION	INTERPRETATION OF THE REGULATION
C08	If a RODAC plate cannot be read because of mould formation or multiple spreaders, for example, this plate is classified as unsuitable.	This plate must not be counted when determining the end-result. If a significant amount of mould/yeast is observed on various plates, a comment should be placed on the final report.
C09	If three or more RODAC plates are classified as unsuitable, the whole test is deemed unsuitable.	The sender is notified of this in writing. The HOSOWO participant investigates the reason for this.
C10	The following scheme is used for filling in the hygienogram form: **# CFU (per plate):** / **Score** 0 / 0 1-40 / 1 41-120 / 2 > 120 / 3	
C11	The appropriate hygienogram form is fully completed (a specimen form is provided further on in this annex).	It is permissible to use a different form as long as all the information and comments are stated. The information of part A on the specimen form is obtained from the HOSOWO authority responsible for sampling.
C12	The end-result is calculated by adding up the relevant scores and dividing them by the number of scores/plates included.	This forms the average score for the RODAC plates concerned.
C13	The end-result is rounded off to one decimal place.	
C14	The result of the negative sample (unsampled plate) is shown on the result form.	
C15	If the negative sample shows growth, the whole hygienogram is regarded as unsuitable.	Negative sample: unsampled plate. The sender is notified of this in writing.

STANDARD	REGULATION	INTERPRETATION OF THE REGULATION
C16	The result of the positive sample (inside the farm gates but not in the barn) is stated on the result form.	
C17	If the positive sample shows no obvious growth, the whole hygienogram is regarded as unsuitable.	Positive sample: plate sampled outside barn. The sender is notified of this in writing
C18	The poultry farmer is informed of the result as soon as possible. This is done by means of a completed hygienogram form or a similar form.	As soon as possible = within 7 working days after sampling. The authority can demonstrate this by means of a copy of the hygienogram form.
C19	The hygienogram form must be stamped with the HOSOWO authority's address or printed on letter paper with address details.	
C20	The hygienogram form must bear the name and signature of the HOSOWO employee responsible at management level for the performance of the HOSOWO activities.	If results are sent electronically, it is sufficient to sign the form digitally or to attach a digital signature. Employee may also be designated by the board.

HYGIENOGRAM FORM FOR FLOOR BARNS

A (information to be obtained from / completed by the HOSOWO authority responsible for hygienogram sampling)

Name of poultry farmer	.....	Name of sampler	.....
Business address of poultry farmer	.....	Business name of sampler	.....
Postcode/place of poultry farmer	.....	Date and start time of sampling	.....
KIP number	.....		
Barn number	.....		

B (to be completed by the HOSOWO authority responsible for hygienogram analysis)

	Sampling sites	1	2	3	4	5	6
1	**Floor**						
2	**Feeding system**					N/A	N/A
3	**Drinking system**			N/A	N/A	N/A	N/A
4	**Wall**						N/A
5	**Ceiling**			N/A	N/A	N/A	N/A
6	**Inlet, inside**			N/A	N/A	N/A	N/A
7	**Feed hopper**		N/A	N/A	N/A	N/A	N/A
END RESULT: ..... ……							

The end-result is determined by dividing the sum of the individual samples by the number of samples taken (the positive and negative samples are not included in the end-result).

Sample check	Result
Positive sample	
Negative sample	

Signature of responsible employee
HOSOWO authority
(approved for hygienogram analysis): _____

Name: _____

HYGIENOGRAM FORM FOR AVIARY AND FREE RANGE BARNS

A (information to be obtained from / completed by the HOSOWO authority responsible for hygienogram sampling)

Name of poultry farmer ……

Business address of poultry farmer ……

Postcode/place of poultry farmer ……

KIP number ……

Barn number ……

Name of sampler ……

Business name of sampler ……

Date and start time of sampling ……

B (to be completed by the HOSOWO authority responsible for hygienogram analysis)

	Sampling sites	1	2	3	4	5	6
1	**Floor**						
2	**Feeding system**				N/A	N/A	N/A
3	**Drinking system**			N/A	N/A	N/A	N/A
4	**Wall**				N/A	N/A	N/A
5	**Ceiling**			N/A	N/A	N/A	N/A
6	**Inlet, inside**			N/A	N/A	N/A	N/A
7	**Feed hopper**		N/A	N/A	N/A	N/A	N/A
8	**Laying nest (if present)**			N/A	N/A	N/A	N/A
9	**Egg belt (if present)**			N/A	N/A	N/A	N/A
10	**Slat (if present)**			N/A	N/A	N/A	N/A
END RESULT: ..... ……							

The end-result is determined by dividing the sum of the individual samples by the number of samples taken (the positive and negative samples are not included in the end-result).

Sample check	Result
Positive sample	
Negative sample	

Signature of responsible employee
HOSOWO authority
(approved for hygienogram analysis): _____

Name: _____

HYGIENOGRAM FORM FOR CAGE/COLONY HOUSING

A (information to be obtained from / completed by the HOSOWO authority responsible for hygienogram sampling)

Name of poultry farmer ……

Business address of poultry farmer ……

Postcode/place of poultry farmer ……

KIP number ……

Barn number ……

Name of sampler ……

Business name of sampler ……

Date and start time of sampling ……

B (to be completed by the HOSOWO authority responsible for hygienogram analysis)

	Sampling sites	1	2	3	4	5	6
1	**Path between cages**				N/A	N/A	N/A
2	**Feeding system**				N/A	N/A	N/A
3	**Drinking system**			N/A	N/A	N/A	N/A
4	**Cage wall**			N/A	N/A	N/A	N/A
5	**Wall**			N/A	N/A	N/A	N/A
5	**Ceiling**			N/A	N/A	N/A	N/A
6	**Inlet, inside**			N/A	N/A	N/A	N/A
7	**Feed hopper**		N/A	N/A	N/A	N/A	N/A
8	**Cage floor**			N/A	N/A	N/A	N/A
9	**Laying nest (if present)**			N/A	N/A	N/A	N/A
10	**Egg belt (if present)**			N/A	N/A	N/A	N/A
END RESULT: ..... ……							

The end-result is determined by dividing the sum of the individual samples by the number of samples taken (the positive and negative samples are not included in the end-result).

Sample check	**Result**
Positive sample	
Negative sample	

Signature of responsible employee
HOSOWO authority
(approved for hygienogram analysis): _____

Name: _____

ANNEX 1.1D REGULATIONS FOR SAMPLING BARN INSPECTION

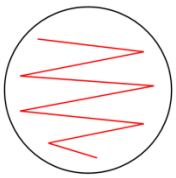
A. introduction

The samples referred to in this annex are taken after a Salmonella infection has been identified in a flock. The samples are taken after the barn has been cleaned and disinfected. The aim is to reveal any Salmonella still present. This annex describes the sampling method and the method to be used to send the samples to a laboratory approved by the national government. The sections in italics in this appendix serve as guidance for the HOSOWO participant.

The samples taken after a Salmonella infection, as described in this annex, are taken in (newly) cleaned and disinfected barns. Make sure that it is safe to enter the barn; there may be some disinfectant residues still present. As a guideline, the barn was ventilated for at least two hours before sampling and the barn was cleaned and/or disinfected the day before sampling.

B. Method

1. The sampler puts on gloves or washes his/her hands before taking the samples.
2. The packaging of the swabs is opened and closed in the barn.
3. The swabs (cotton buds) are moistened in physiological saline solution or buffered peptone water.
   The swabs themselves must also be analysed (not only the dirt picked up on them). So be sure to use clean/sterile swabs.
4. A total of at least 50 swabs are taken.
5. These swabs are taken at critical (and visibly dirty) sites.
   Critical sites include cracks, tears, (connecting) joints, places that are difficult to clean/disinfect, (inside of) feeding and watering lines and ventilation points. To identify these sites, it is advisable to first walk round the barn.
6. Sampling is done as follows:
   a. If possible, the swab is wiped over a sampling surface the size of a RODAC plate (5.5 cm).
      Use a zig-zag motion in contiguous lines.
   b. The swabs are collected in two collection units (2 x 25 swabs).
      For example, the swabs are collected in a plastic pot.
   c. By way of derogation from point 6b, in the case of an extensive tracing test for Salmonella infection, a different collection method may also be used. However, no more than 25 swabs may be collected per collection unit.
   d. The collection units must be closed immediately after filling.



7. The samples are sent to the laboratory within 24 hours.
   The analysis must be started no later than the second day after sampling.
8. The HOSOWO authority is responsible for sending/transporting the samples.
   This must not be left to the operator/poultry farmer or another similar party.
9. The samples are sent to a laboratory approved by the country concerned.

10. The following details must be clearly stated in or on the package:
    a. Name and address
    b. Poultry farmer's KIP number and activity (animal species)
    c. Type of sample (i.e. swab)
    d. Barn number (per sample)
    e. Date of sampling and start time of sampling (hour);
    f. Type of test (regular test for Salmonella)
    g. Sampler (= HOSOWO authority)
    h. Name and signature of party submitting the sample;
    i. Date
11. The samples are packed in accordance with current, generally accepted packing instructions. *Ensure that no leakage or cross-contamination can occur.*
12. The samples shall be sent to the laboratory no later than the working day following the day of sampling. The transport of the samples takes place by express service or courier service at a temperature between 0˚C and 25˚C, avoiding exposure to sunlight and excessive heat. *The authority can demonstrate this by placing a min-max thermometer and requesting a logbook from the courier service. The minimum and maximum temperature are noted in the logbook.*

ANNEX 1.1E REGULATIONS FOR SAMPLING DRINKING WATER TEST

A. Introduction

A drinking water test safeguards the quality of the drinking water for poultry. This annex describes the sampling sites. The samples are taken at a time that animals are present in the barn. The sampling for a drinking water test is performed by a HOSOWO authority or by the veterinarian with whom the poultry farmer has an agreement.

For the chemical and bacteriological parameters, the standards as set out in the IKB KIP and/or IKB Ei quality regulations are applied.

B. Method

1. The sample for bacteriological and chemical testing is taken at the end of the system of pipes at the final drinking/tapping point.
2. The sampler washes and disinfects his/her hands before taking the samples or puts on gloves before taking the sample.
3. The sampler uses only sterile pots.
4. The inside of the lid and the inside of the pot are not touched with the hands and do not touch the drinking point/nipple.
5. The sampler wipes the outside of the drinking point/tapping point with a (nipple) cloth to remove any traces of feed, manure and/or feathers from the drinking point. The sampler then drains off 1L water. The sampler does not close the tap/drinking point and continuously taps the sample.
6. After the pot has been filled, the lid is screwed firmly onto the pot to prevent leakage/contamination.
7. Each sample contains at least 150 ml of water.
8. A label that states at least the name of the poultry farmer and the KIP number must attached to each sample.
9. A submission form that states at least the following information must attached to each sample: Name and address details of the poultry farmer, name and organisation of sampler, date of sampling and time of sampling (hour), KIP number and barn number .
10. The sampler is responsible for sending the sample to the authority concerned that performs the analysis.
11. Sending/storage of the samples must take places as soon as possible and cooled (max. 7 ˚C) and not exposed to light.