COMMISSION DECISION

of 20 December 2007

concerning a financial contribution from the Community towards a survey on the prevalence of *Salmonella* spp. and Methicillin-resistant *Staphylococcus aureus* in herds of breeding pigs to be carried out in the Member States

(notified under document number C(2007) 6579)

(2008/55/EC)

THE COMMISSION OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Community,

Having regard to Council Decision 90/424/EEC of 26 June 1990 on expenditure in the veterinary field [1], and in particular Article 20 thereof,

Whereas:

(1) Decision 90/424/EEC lays down procedures governing a financial contribution by the Community towards specific veterinary measures, including technical and scientific measures. It provides for the Community to undertake or assist Member States in undertaking the technical and scientific measures necessary for the development of Community veterinary legislation and for the development of veterinary education or training.

(2) Under Article 4 and Annex I of Regulation (EC) No 2160/2003 of the European Parliament and of the Council of 17 November 2003 on the control of *Salmonella* and other specified food-borne zoonotic agents [2], a Community target is to be established for reducing the prevalence of *Salmonella* in populations of herds of breeding pigs.

(3) The Task Force on Zoonoses Data Collection of the European Food Safety Authority (EFSA) adopted on 30 April 2007 a Report on a proposal for technical specifications for a baseline study on the prevalence of *Salmonella* in breeding pigs [3] (the *Salmonella* report).

(4) In order to set the Community target for the reduction of the prevalence of zoonoses and zoonotic agents as foreseen in Article 4 of Regulation (EC) No 2160/2003 and to consider the best approach to evaluate in the future the achievement of such target, comparable data on the percentage of *Salmonella* infected holdings of breeding pigs in the Member States needs to be available. Such information is not available and a special survey should therefore be carried out to monitor the prevalence of *Salmonella* in breeding pigs over a suitable period in order to take account of possible seasonal variations. The survey should be based on the *Salmonella* report.

(5) The *Salmonella* report also recommends additional sampling for the estimation of within-holding prevalence. Such sampling should be carried out by a number of Member States geographically representing the different situations in the Community.

(6) Meticilline-resistant *Staphylococcus aureus* (MRSA) infections have been recognised as an important risk in hospitals for several decades. MRSA is resistant to the most commonly used antibiotics and it is particularly dangerous for patients with a decreased immunity. The number of deaths attributed to MRSA in the United Kingdom has been estimated at around 3 000 per year. The costs for treatment per patient are estimated at EUR 12 000 to 15 000. Additional expenses are incurred due to hygiene and control programmes to prevent or limit the infection in hospitals.

(7) A new strain of MRSA (ST398) has recently been detected in production animals in several Member States. In particular, pigs have been recognised as an important source of infection for pig farmers or their relatives by direct contact with pigs. Infection with the new strain may also enter hospitals as previously MRSA did in several Member States.

(8) In order to increase awareness and to assess whether it is necessary to take measures to detect and control MRSA in order to reduce their prevalence and the risk they pose to public health, comparable data on the percentage of MRSA (ST398) infected holdings of breeding pigs in the Member States are needed. Such information is not available and a special survey should therefore be carried out to monitor the prevalence of MRSA in breeding pigs over a suitable period in order to take account of possible seasonal variations.

[1] OJ L 224, 18.8.1990, p. 19. Decision as last amended by Regulation (EC) No 1791/2006 (OJ L 363, 20.12.2006, p. 1).
[2] OJ L 325, 12.12.2003, p. 1. Regulation as last amended by Commission Regulation (EC) No 1237/2007 (OJ L 280, 24.10.2007, p. 5).
[3] *The EFSA Journal* (2007) 99, 1-28.

(9) The Task Force on Zoonoses Data Collection of the EFSA adopted on 19 November 2007 a Report including a proposal for technical specifications for a baseline survey on the prevalence of Methicillin-Resistant *Staphylococcus aureus* (MRSA) in breeding pigs (the MRSA report) [1]. The MRSA report makes recommendations with regard to the sampling frame, sample collection protocol, laboratory analytical methods and reporting. The technical specifications for the survey provided for in this Decision should be based on that report.

(10) In accordance with Commission Decision 2007/636/EC of 28 September 2007 concerning a financial contribution from the Community towards a survey on the prevalence of *Salmonella* spp. in herds of breeding pigs to be carried out in the Member States [2], Member States are to carry out a survey in herds of breeding pigs from 1 January 2008 until 31 December 2008 in order to assess the prevalence of *Salmonella* spp. Taking into account the public health significance of MRSA, the emerging risk of pigs as source of infection for humans and the lack of comparable information on the prevalence of MRSA in herds of breeding pigs in different Member States, additional sampling during the survey provided for in Decision 2007/636/EC is the most rapid and cost-effective way to evaluate the prevalence of MRSA in herds of breeding pigs in the Community.

(11) The survey is to provide technical information necessary for the development of Community veterinary legislation as appropriate. Given the importance of collecting comparable data on the prevalence of MRSA in breeding pigs in the Member States, the Member States should be granted a Community financial contribution for implementing the specific requirements of the survey. It is appropriate to reimburse 100 % of the costs incurred on the purchase of swabs and the laboratory testing, subject to a ceiling. All other costs incurred, such as costs for sampling, travelling and administration, should not be eligible for any Community financial contribution.

(12) The financial contribution from the Community should be granted provided that the survey is carried out in accordance with the relevant provisions of Community law and that certain other conditions are complied with, including transmission of results within prescribed deadlines.

(13) For reasons of administrative efficiency all expenditure presented for a financial contribution by the Community should be expressed in EUR. In accordance with Council Regulation (EC) No 1290/2005 of 21 June 2005 on the financing of the common agricultural policy [3], the conversion rate for expenditure in a currency other than euro should be the rate most recently set by the European Central Bank prior to the first day of the month in which the application is submitted by the Member State concerned. For reasons of clarity and transparency, Decision 2007/636/EC should be repealed and a financial contribution from the Community toward the surveys for the prevalence of *Salmonella* and MRSA should be laid down in this single Decision.

(14) In order to ensure the coherence in carrying out the surveys, this Decision should apply from 1 January 2008, date of application of Decision 2007/636/EC.

(15) The measures provided for in this Decision are in accordance with the opinion of the Standing Committee on the Food Chain and Animal Health,

HAS ADOPTED THIS DECISION:

Article 1

Subject matter and scope

This Decision lays down rules on the financial contribution from the Community towards baseline surveys to be carried out in the Member States to assess the prevalence of *Salmonella* spp. (the *Salmonella* survey) and Methicilline-resistant *Staphylococcus aureus* (MRSA) (the MRSA survey) across the Community in breeding pigs sampled at farm level.

Article 2

Definition

For the purposes of this Decision, 'competent authority' shall be the authority or authorities of a Member State as designated pursuant to Article 3 of Regulation (EC) No 2160/2003.

Article 3

Scope of the surveys

1. The Member States shall carry out the *Salmonella* survey in accordance with Parts A and B of Annex I until 31 December 2008.

2. The Member States shall carry out the MRSA survey in accordance with Parts A and C of Annex I until 31 December 2008.

[1] *The EFSA Journal* (2007) 129, 1-14.
[2] OJ L 257, 3.10.2007, p. 30.
[3] OJ L 209, 11.8.2005, p. 1. Regulation as last amended by Regulation (EC) No 1437/2007 (OJ L 322, 7.12.2007, p. 1).

Article 4

Performance of sampling and analyses

Sampling and analysis shall be performed by the competent authority or under its supervision in accordance with the technical specifications set out in Annex I.

Article 5

Conditions for granting a Community financial contribution

1. The financial contribution from the Community towards the costs of analyses pursuant to this Decision shall be granted to the Member States up to the maximum total amount for co-financing set out in Annex II to this Decision for the duration of the surveys provided for in this Decision.

2. The financial contribution from the Community provided for in paragraph 1 shall be granted to the Member States provided that the *Salmonella* and MRSA surveys are implemented in accordance with the relevant provisions of Community law, including the rules on competition and on the award of public contracts, and subject to compliance with the following conditions:

(a) the national laws, regulations and administrative provisions required to implement the survey must enter into force on the day of application of this Decision at the latest;

(b) a progress report containing the information listed in Part D of Annex I and covering the first three months of the surveys must be submitted to the Commission by 31 May 2008 at the latest.

(c) a final report on the implementation of the surveys, together with supporting evidence for the costs incurred by the Member States for the analyses and the results attained during the period from 1 January 2008 to 31 December 2008 must be submitted to the Commission by 31 March 2009 at the latest;

(d) the surveys must be implemented effectively.

The supporting evidence for the costs incurred as referred to in point (c) of the second paragraph shall comprise at least the information set out in Annex III.

3. If the final report referred to in paragraph 2(c) is submitted after 31 March 2009 but before 30 April 2009, the financial contribution to be paid by the Community shall be reduced by 25 %.

If the final report is submitted after 30 April 2009 but before 31 May 2009, the financial contribution shall be reduced by 50 %.

No financial contribution shall be paid if the final report is submitted after 31 May 2009.

Article 6

Maximum amounts to be reimbursed

1. The maximum amounts of the financial contribution from the Community towards the costs to be reimbursed to the Member States for analyses covered by the *Salmonella* survey shall not exceed the following:

(a) EUR 20 per test for bacteriological detection of *Salmonella* spp.;

(b) EUR 30 for serotyping of the relevant isolates.

2. The maximum amounts of the financial contribution from the Community towards the costs to be reimbursed to the Member States for analyses covered by the MRSA survey shall not exceed the following:

(a) EUR 30 per test for bacteriological detection of MRSA;

(b) EUR 8 per identification of the presence of MRSA by PCR;

(c) EUR 25 per *Staphylococcus* type A typing (Spa typing);

(d) EUR 150 per multi locus sequence typing (MLST) of relevant isolates;

(e) EUR 1,25 per swab.

Article 7

Collection of data, assessment and reporting

1. The competent authority responsible for preparing the yearly national report pursuant to Article 9(1) of Directive 2003/99/EC of the European Parliament and of the Council [1] shall collect and assess the results of the surveys and forward them to the Commission.

[1] OJ L 325, 12.12.2003, p. 31. Directive as amended by Council Directive 2006/104/EC (OJ L 363, 20.12.2006, p. 352).

2. The Commission shall forward the national data and the assessment referred to in paragraph 1 to the European Food Safety Authority, which shall examine them.

3. National data and results shall be made publicly available in a form that ensures confidentiality.

Article 8

Conversion rate for expenditure

Where a Member State's expenditure is in a currency other than euro, the Member State concerned shall convert its expenditure into euro by applying the most recent exchange rate set by the European Central Bank prior to the first day of the month in which the application for the financial contribution from the Community is submitted by the Member State.

Article 9

Repeal of Decision 2007/636/EC

Decision 2007/636/EC is hereby repealed.

Article 10

Application

This Decision shall apply from 1 January 2008.

Article 11

Addressees

This Decision is addressed to the Member States.

Done at Brussels, 20 December 2007.

For the Commission

Markos KYPRIANOU

Member of the Commission

ANNEX I

TECHNICAL SPECIFICATIONS REFERRED TO IN ARTICLE 3, ARTICLE 4 AND ARTICLE 5(2)(b)

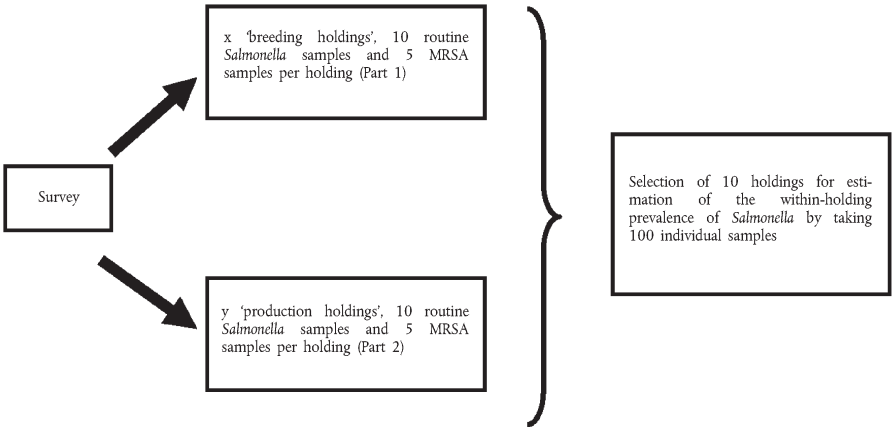
Part A: Overview and sampling frame

1. **Overview of the survey**

The survey shall be performed according to the overview in Figure 1.

Figure 1

Overview of the survey



2. **Sampling frame**

2.1. *The delineation of the population*

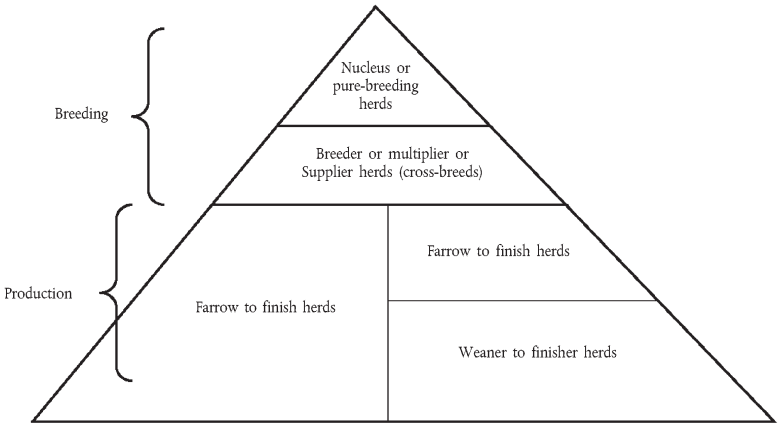
The survey shall be carried out on holdings harbouring at least 80 % of the breeding pig population in a Member State. Preferentially holdings having 50 breeding pigs or more shall be sampled. However, if those holdings having 50 breeding pigs or more do not contain 80 % of the national herd of breeding pigs, then smaller holdings with less than 50 breeding pigs shall also be sampled.

Holdings with breeding pigs shall be classified either as 'breeding holdings' or as 'production holdings'. Breeding holdings sell gilts and/or boars for breeding purposes. Typically, they sell 40 % or more of the gilts that they rear for breeding whilst the remainder are sold for slaughter. In contrast, production holdings mainly sell pigs for fattening or slaughter.

The *Salmonella* and MRSA prevalence must be measured separately in breeding holdings (Part 1 of the *Salmonella* and MRSA surveys) and in production holdings (Part 2 of the *Salmonella* and MRSA surveys), representing the herds as indicated in Figure 2, but excluding weaner to finisher herds.

Figure 2

Overview of holdings



2.2. *The sample and the sampling strategy*

Both parts of the *Salmonella* and MRSA surveys shall have a similar two-stage sampling design. In the first stage, a random sample of holdings shall be selected in every Member State from the breeding holdings and a second random sample shall be selected from the production holdings group. The number of holdings required is discussed in section 2.3. In the second stage, a number of pens shall be selected for sampling within every selected holding (see Section 2.2.2).

2.2.1. First stage: selection of holdings

Each Member State must create two sampling frames. The first shall list all eligible breeding holdings (usually, those holdings with at least 50 breeding pigs — see Section 2.1) and the second shall list all eligible production holdings. The required number of holdings for each part of the *Salmonella* and MRSA surveys will then be selected at random from each list. A random sample is intended to ensure that the surveys include holdings with a range of herd sizes and from all regions of a Member State where pig-keeping is practised. It is recognised that in some Member States, there may be a few holdings (e.g. fewer than 10 % of all eligible holdings) with a very large herd size. Thus, random selection may by chance result in none of these very large herds being selected. A Member State may use a stratification criterion prior to holding selection — for example, to define a stratum containing the 10 % of largest herds and to allocate 10 % of the required sample size to this stratum. Similarly, a Member State may stratify the sample across administrative regions according to the proportion of eligible herds within each region. Any stratification that is considered should be described in the report that a Member State submits to the Commission in accordance with Part D (1).

If a selected holding cannot be sampled (for example, if it no longer exists when sampling is carried out) a new holding shall be selected at random from the same sampling frame. If any stratification (e.g. on herd size or region) was in operation, then the new holding should be selected from the same stratum.

The primary sample size (number of holdings to be sampled) shall be approximately equally distributed over the year to cover the different seasons, as far as possible. Samples shall be taken from approximately one twelfth of the number of holdings each month.

Outdoor holdings must be included in the survey but there shall be no mandatory stratification on this production type.

2.2.2. Second stage: sampling on the holding

In each selected breeding herd and production herd the pens, yards or groups of breeding pigs over six months of age to be sampled will be randomly selected.

The number of pens, yards, or groups to be sampled must be proportionally allocated according to the numbers of breeding pigs in the different stages of production (pregnant, non-pregnant, and other categories of breeding pigs). The exact age categories to be sampled are not prescribed, but this information shall be collected during the sampling.

Breeding pigs that have arrived recently to the herd and are held in quarantine shall not be included in the *Salmonella* and MRSA surveys.

2.3. *The sample size calculation*

2.3.1. Primary sample size (first-stage sample size)

A regular primary sample size calculation shall be conducted for the breeding holdings and a second regular primary sample size calculation shall be conducted for the production holdings. The primary sample size shall be the number of breeding holdings to be sampled and the number of production holdings to be sampled in each Member State and it shall be determined taking into account the following criteria, assuming simple random sampling:

(a) the total number of breeding holdings (breeding holdings, Part 1 of the *Salmonella* and MRSA surveys);

(b) the total number of production holdings (production holdings, Part 2 of the *Salmonella* and MRSA surveys);

(c) annual expected prevalence (p): 50 %;

(d) desired confidence level (Z): 95 %, corresponding to a Z_α value of 1,96;

(e) accuracy (L): 7,5 %;

(f) using these values and the formula:

$$n_\infty = \frac{(Z_\alpha)^2\, p(1-p)}{L^2}$$

The calculation shall be conducted firstly, for the breeding holdings and secondly, for the production holdings. In each case, the assumptions in steps c-e above are the same.

For practical purposes, if there are 100 000 or more holdings in either the breeding herds sampling frame or the production herds sampling frame then that population can be considered to be infinite and the number of holdings to be randomly selected from that sampling frame is 171 (see Table 1). Where the number of breeding herds or production herds is less than 100 000 a finite population correction factor is applied and fewer holdings need to be sampled as shown in Table 1.

As an example, if there are in a Member State 1 000 holdings belonging to the production holding group and 250 holdings belonging to the breeding holding group, 147 holdings must be sampled in the production holding group and 102 holdings must be sampled in the breeding holding group.

Table 1

Number of holdings with breeding pigs to sample in either part of the *Salmonella* and MRSA surveys as a function of the finite population size (total number of holdings with breeding pigs in the Member States)

Number of holdings with breeding pigs (N)	Sample size (n) for finite population size 7,5 % accuracy
100 000	171
10 000	169
5 000	166
2 000	158
1 000	147
500	128
250	102
150	80
125	73
100	64
90	59
80	55
70	50
60	45
50	39
40	33
30	26
20	18
10	10

Non-response shall be anticipated e.g. by increasing the sample size in each group by 10 %. Any unsuitable holding shall be replaced in the process of the *Salmonella* and MRSA surveys (see Section 2.2.1).

In case an estimation of the number of 'breeding holdings' is not possible prior to the start of the survey, a number of holdings shall be selected for sampling as in Table 1 based on the total number of holdings with sows (X holdings). The number of holdings to be sampled shall be increased by at least 30 % ((X + 30 %) holdings). Prior to the survey, the competent authority shall identify a number of breeding holdings, equal to at least this additional 30 %. While visiting the farms, the holding will be classified as breeding or production holding according to the definitions above.

2.3.2. Secondary sample size (second-stage sample size)

In each selected holding samples shall be collected from 10 randomly chosen pens, yards, or groups of breeding pigs. If necessary (for example, in farrowing accommodation or where sows are kept in small groups of less than 10 individuals), a group can consist of more than one pen. At least 10 individual breeding pigs should contribute to each routine *Salmonella* sample.

However, where on smallholdings or holdings with large numbers of breeding pigs kept outdoors in paddocks, the number of pens, yards or groups is less than 10, sampling of the same pen, yard, or group shall be required so that a total of 10 routine *Salmonella* samples are submitted.

Part B: Sample collection and analysis for the *Salmonella* survey

1. Sample collection in the herds

1.1. *Type and detail of the routine sample*

The material collected for bacteriological analysis shall be freshly voided faeces representing the whole holding, which is the unit of interest. Since every holding is unique, it shall be decided, before starting the sampling, which pens, yards, or groups within the holding are sampled. The sample collected shall be placed in a separate container avoiding cross-contamination and sent to the laboratory.

Each pooled sample shall total at least 25 g and two approaches may be employed to collect these pooled faeces samples:

1. where there is an accumulation of mixed faeces within an area of a pen or yard, a large swab (e.g. 20 cm × 20 cm) can be used to pass through the faecal mass, ensuring that at least 25 g of mixed material is collected. This may be achieved by for example, moving the swab along a 2-metre zig-zag path such that it is well coated with faecal material. If necessary, for example due to hot weather or on slatted flooring, then the swab may be moistened with an appropriate liquid such as drinking water.

2. where there is no such accumulation, for example in a field, large yard, in a farrowing house, or pens or other accommodation with low numbers of pigs per group, then individual pinches shall be selected from individual fresh faecal masses or places so that a minimum of 10 individuals, contribute to a total sample volume of at least 25 g. The sites from which these pinches are collected should be distributed in a representative manner across the area concerned.

Approach 1 shall be preferred where practical. In this approach at least 10 individual pigs must contribute to each sample taken, otherwise approach 2 shall be applied.

1.2. *Additional sampling for the within-holding prevalence study*

Together, 10 holdings, selected at random from the overall sample of breeding holdings and of production holdings are subjected to more intensive sampling. On these holdings 10 routine samples shall be collected in the same manner as described previously (Section 2.1 of Part A). In addition, 10 individual samples of at least 30 g shall be collected in each selected pen and shall be identified in such a manner that these 10 individual samples can be associated with the routine sample from that pen. Thus in total, 10 routine samples and 100 (10 × 10) individual samples shall be collected from each of these 10 holdings. The processing of these samples is described in Section 2.3.1.

This sampling should be applied in Czech Republic, Denmark, Romania, Slovenia, Sweden and the United Kingdom.

1.3. *Sample information*

All relevant information available from the sample shall be recorded on a sampling form produced by the competent authority to enable the data requirements in Part D to be fulfilled.

Each sample and its sample form shall be labelled with a unique number which shall be used from sampling to testing, and with the code of the pen. The competent authority must arrange for the issue and use of a unique numbering system.

1.4. *Transport of samples*

Samples shall be preferably kept between + 2 and + 8 °C and free of external contamination during transportation. The samples shall be sent to the laboratory as quick as possible within 36 hours by fast mail or courier and shall reach the laboratory no later than 72 hours after sampling.

2. Laboratory analytical methods

2.1. *Laboratories*

Analysis and serotyping shall take place at the National Reference Laboratory (NRL). Where the NRL does not have the capacity to perform all analyses or if it is not the laboratory that performs detection routinely, the competent authorities may decide to designate a limited number of other laboratories involved in official control of *Salmonella* to perform the analyses. These laboratories shall have proven experience of using the required detection method and have a quality assurance system complying with ISO 17025 and be submitted to the supervision of the NRL.

2.2. *Receipt of samples*

At the laboratory, samples shall be kept refrigerated until bacteriological examination, which shall preferably be carried out within 24 hours after receipt but in any case no later than 96 hours after the sample was collected.

2.3. *Sample analysis*

Member States shall guarantee that all involved parties have been sufficiently trained to carry out the analyses.

2.3.1. Preparation

In the laboratory, routine samples shall be mixed carefully and thoroughly, before 25 g is collected for analysis.

For evaluation of the within-holding prevalence in accordance with paragraph 1.2, each of the individual collected samples (30 g) needs to be divided into two parts. One part, weighing at least 25 g shall be mixed carefully and thoroughly and subsequently cultured individually. The remaining second part is to be used to prepare an artificially pooled sample from the 10 individual samples in the selected pen, group or yard. This latter part shall be prepared by adding 10 times 2,5 g of the individual samples to create an artificially pooled sample of 25 g. The artificially pooled samples are mixed carefully and thoroughly before analysis. In total, 10 routine samples, 10 artificially pooled sample and 100 individual samples shall be analysed from each of the 10 holdings selected for the estimation of the within-holding prevalence.

2.3.2. Detection and identification methods

2.3.2.1. Detection of *Salmonella*

The method recommended by the Community Reference Laboratory (CRL) for *Salmonella* in Bilthoven, the Netherlands, shall be used. This method is described in the Annex D of ISO 6579: 'Detection of *Salmonella* spp. in animal faeces and in samples of the primary production stage'. The latest version of Annex D shall be used.

2.3.2.2. Serotyping of *Salmonella*

All strains isolated and confirmed as *Salmonella* spp. shall be serotyped according to the Kaufmann-White scheme, by the NRL for *Salmonella*.

For quality assurance, 16 typable strains and 16 non-typable isolates shall be sent to the CRL for *Salmonella*. A proportion of these isolates shall be sent to the CRL on a quarterly basis. If fewer strains have been isolated, all shall be sent.

2.3.2.3. Phage typing of *Salmonella*

In case isolates of *Salmonella Enteritidis* and *Salmonella Typhimurium* are phage typed (optional), the methods described by the WHO reference centre for phage typing of *Salmonella* of the Health Protection Agency (HPA) Colindale, London, shall be used.

Part C: Sample collection and analysis for the MRSA survey

1. Type and detail of sample

1.1. *Sample collection*

Five dust samples shall be gathered using five dry sterile swabs of about 500 cm^2 each from five of the 10 pens selected for sampling under part A. These five pens shall be chosen in a way that breeding pigs in different production stages are included. For each pen dorsal surfaces of pen partition walls shall be swabbed. In case there is not enough dust present, then ventilator ducts etc. shall be sampled in addition. After use, the soiled swab shall be placed in a sterile plastic bag.

The creation of aerosol in the building shall be avoided during sampling.

1.2. *Sample information*

Each sample and its sample form shall be labelled with a unique number which shall be used from sampling to testing. The competent authority shall arrange for the issue and use of a unique numbering system.

1.3. *Transport of samples*

Samples shall be kept at constant temperature between + 2 °C and 25 °C (room temperature) and free of external contamination during storage and transportation. The samples shall be sent to the laboratory as quickly as possible and reach the laboratory no later than 10 days after sampling.

2. Laboratory analytical methods

2.1. *Laboratories*

Analysis and subtyping of MRSA shall take place in experienced laboratories. This shall preferably be the National Reference Laboratories (NRL's) for *Staphylococcus aureus* and/or antimicrobial resistance in the Member States. In case the NRL does not have the capacity or experience to perform the analyses or if it is not the laboratory that performs detection routinely, the competent authority shall decide to designate other experienced laboratories or a NRL in another Member State to perform the analyses. These laboratories shall have proven experience of using the required methods and have an accreditation system in place according to ISO 17025. An up-to-date list of authorised laboratories can be consulted on the website of the Community Reference Laboratory for antimicrobial resistance (CRL-AR) in Copenhagen, Denmark.

2.2. *Receipt of samples*

Samples arriving 10 days after sampling shall be discarded unless bacteriological examination can be started within 13 days. At the laboratory, samples shall be kept at a constant temperature between + 2 °C and 25 °C until bacteriological examination, which shall be carried out within 13 days after sampling.

2.3. *Sample analysis*

2.3.1. Selective enrichment

In the laboratory the five dust swabs shall be pooled in a 100 ml of Mueller-Hinton broth supplemented with 6,5 % NaCl and incubated at 37 °C for 16-20 h. One millilitre of this shall then be inoculated into 9 ml Tryptone Soy Broth + 3,5 mg/l cefoxitin and 75 mg aztreonam and shall be incubated for a further 16-20 h at 37 °C. One loop-full of this shall then be spread onto a chromogenic agar selective for MRSA and incubated for 24-48 h at 37 °C. The specific agar recommended by the CRL-AR shall be used. This agar is described in the CRL-AR website.

Based on colony morphology and colour, up to five colonies indicative for being MRSA shall be subcultivated on blood agar. Presumptive *Staphylococcus aureus (S. aureus)* shall at this stage either be stored under appropriate conditions (– 80 °C) for later identification and characterisation or processed immediately.

2.3.2. Identification of MRSA

Presumptive *S. aureus* shall be identified as *S. aureus* and MRSA by PCR. The identification shall be performed using a multiplex PCR with simultaneous identification of the mecA-gene or two different PCR shall be performed. To limit the amount of work only one of the five presumptive *S. aureus* isolates shall initially be identified. If this isolate is identified as MRSA, it shall be stored. No further testing of the remaining four isolates is required if the first isolate is identified as MRSA and they can be discarded. If the first isolate is not identified as MRSA, the next of the initial five isolates shall be tested. This process shall continue until one MRSA has been identified or all five isolates have been tested. Alternatively, identification by PCR as a first step can be done on a pool of the five presumptive colonies from a sample. In case of a positive PCR, the analysis shall be repeated on individual colonies to identify a positive colony.

For quality assurance, 16 presumptive *S. aureus* isolates which were not identified as MRSA, as well as 16 MRSA strains, sampled over the whole year 2008 shall be sent to the CRL-AR. A proportion of these isolates shall be sent to the CRL-AR on a quarterly basis. In case less than 16 isolates were verified as MRSA all these isolates shall be sent.

2.3.3. Subtyping for possible link to human isolates

Positive MRSA's shall be tested for *Staphylococcus* type A (Spa-typing). The typing shall be performed at the NRL or under its supervision, or isolates shall be forwarded to the CRL-AR, which shall then perform the typing.

On a subset of representative isolates (about 2 % of the number of pooled samples) MLST-typing shall be performed by the NRL or the CRL-AR.

2.3.4. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing is optional. If carried out, MRSA isolates shall be tested for antimicrobial susceptibility using micro-dilution at least to the following antimicrobial agents: ciprofloxacin, erythromycin, fusidic acid, gentamicin, linezolide, mupirocin, sulphametoxazole, trimethoprim, tetracycline, chloramphenicol, vancomycin and quinupristin/dalfopristin. Reporting on antimicrobial susceptibility shall be carried out in accordance with Article 9(1) of Directive 2003/99/EC.

2.4. *Storage of isolates*

The isolates shall be stored at the National Reference Laboratories (NRL's) using the method for NRL culture collection ensuring viability and no changes in properties of the strains for a minimum of five years. This is in order to allow, for instance, later testing for antimicrobial susceptibility or other types of characterisation. Also isolates sent to the CRL-AR shall be stored for a minimum of five years. Isolates shall be stored under conditions not allowing changes in properties (– 80 °C). If the laboratory in charge do not have the available storage capability, isolates shall be forwarded to the CRL-AR, which shall store these isolates.

3. **Reporting from the laboratories**

All analytical results shall be sent on a confidential basis only from the laboratory to the competent authority of the Member States where the dust samples were collected.

Part D: Reporting from the Member States

1. **Overall description on the implementation of the *Salmonella* and MRSA surveys**

A report in text format shall include at least:

(a) Member State;

(b) a description of the population of holdings with breeder pigs:

1. breeding holdings:

(i) total number of breeder holdings;

(ii) total number of nucleus holdings;

(iii) total number of multiplier holdings;

(iv) number of breeder holdings planned to be sampled, and number of breeder holdings actually sampled; number of holdings planned for sampling but not sampled and the reason therefore;

(v) comments on the overall representativeness of the breeding holdings sampling programme;

2. production holdings:

(i) total number of production holdings;

(ii) total number of farrow to weaner/grower holdings;

(iii) total number of farrow to finish holdings;

(iv) number of production holdings planned to be sampled, and number of production holdings actually sampled; number of holdings planned for sampling but not sampled and the reason therefore;

(v) possible comment on overall representativeness of the production holdings sampling programme;

(c) number of samples from the *Salmonella* survey obtained and analysed:

(i) from breeding holding;

(ii) from production holdings;

(iii) from holdings sampled for within-holding prevalence study;

(d) overall results from the *Salmonella* survey:

(i) prevalence of breeding holdings and of production holdings infected with *Salmonella*, and serovars of *Salmonella*;

(ii) outcome of within-holding prevalence study;

(e) list of laboratories responsible in the *Salmonella* survey for:

(i) detection;

(ii) serotyping;

(iii) phage typing (if carried out).

(f) number of samples from the MRSA survey obtained and analysed:

(i) from breeding holding;

(ii) from production holdings;

(g) overall results from the MRSA survey: prevalence of breeding holdings and of production holdings infected with MRSA, based on detection and confirmation by PCR;

(h) list of laboratories responsible in the MRSA survey for:

(i) detection;

(ii) PCR;

(iii) Spa typing;

(iv) MLST typing.

2. **Complete data on each holding sampled and corresponding tests results:**

The Member States shall submit the results of the *Salmonella* and MRSA surveys electronically to the Commission in the form of raw data using a data dictionary and data collection requirements established and provided by the Commission.

2.1. *Information on the holding*

The following information shall be collected in Member States and transmitted to the Commission for each holding selected for sampling:

(a) code of the holding;

(b) holding production type:

(i) indoor versus 'any stage of the production kept outdoors';

(ii) nucleus, multiplier, farrow to weaner, farrow to finish and farrow to grower;

(c) holding size: the number of breeding pigs present at the time of sampling (adult inventory);

(d) replacement policy: all replacement breeding pigs purchased; some replacement breeding pigs homebred or all replacement breeding pigs homebred;

(e) (voluntarily) clinical symptoms of diarrhoea: were there symptoms of diarrhoea within the three months before the sampling?

2.2. *Information on all samples collected within the Salmonella survey*

The following information shall be collected in Member States for each sample sent to the laboratory within the frame of the *Salmonella* survey:

(a) code of the sample;

(b) code of the laboratory involved in initial analysis;

(c) date of sample collection;

(d) date laboratory analysis begun;

(e) detection of *Salmonella*: qualitative result (positive/negative);

(f) serotyping of *Salmonella*: serovar(s) detected (may be more than one);

(g) age of the pigs: all gilts versus mixed age breeding pigs;

(h) sex: only sows; sows and boars or only boars;

(i) production stage: maternity; mating, gestation (other?);

(j) housing: slatted floor (fully/partly); solid floor; deep straw or other;

(k) diet: are pigs in this pen, yard or group fed compound feed exclusively;

(l) feed supplement: is there any *Salmonella* reducing substance added to the feed (like organic acid, a probiotic);

(m) systematic use of antibiotics: are antibiotics used in all animals of this group by any route of administration;

(n) the last date of administration of antimicrobials to the animals (within the last four weeks).

2.3. *Additional information on the samples collected within the Salmonella survey for the within holding prevalence*

The following addition information shall be collected in Member States for each individual sample sent to the laboratory within the frame of the sampling for the within-holding prevalence:

(a) code of the pooled sample;

(b) detection of *Salmonella* in each individual sample: qualitative result (positive/negative);

(c) serotyping of *Salmonella* in each individual sample: serovar(s) detected (may be more than one).

2.4. *Information on the samples collected within the MRSA survey*

The following information shall be collected in Member States and for each sample sent to the laboratory:

(a) the code of the sample;

(b) the code/name of the laboratory involved in the detection;

(c) the date of sample collection;

(d) the date laboratory analysis begun;

(e) the result of detection of MRSA (positive/negative);

(f) the code/name of the laboratory involved in the PCR;

(g) the result of PCR;

(h) the code/name of the laboratory involved in the Spa typing;

(i) the result of Spa typing;

(j) code/name of the laboratory involved in the MLST-typing,

(k) the result of MLST typing.

ANNEX II

MAXIMUM COMMUNITY FINANCIAL CONTRIBUTION TO THE MEMBER STATES REFERRED TO IN ARTICLE 5

Member State	Maximum total amount for co-financing of analyses (EUR)
Belgium – BE	74 003
Bulgaria – BG	64 672
Czech republic – CZ	120 621
Denmark – DK	114 829
Germany – DE	71 750
Estonia – EE	11 583
Ireland – IE	53 732
Greece – EL	48 584
Spain – ES	102 317
France – FR	102 317
Italy – IT	98 134
Cyprus – CY	24 775
Latvia – LV	4 183
Lithuania – LT	17 053
Luxembourg – LU	14 801
Hungary – HU	92 021
Malta – MT	0
Netherlands – NL	107 786
Austria – AT	73 037
Poland – PL	105 212
Portugal – PT	67 889
Romania – RO	126 734
Slovenia – SI	93 594
Slovakia – SK	66 924
Finland – FI	80 116
Sweden – SE	93 594
United Kingdom – UK	120 621
Total	1 950 878

ANNEX III

CERTIFIED FINANCIAL REPORT ON THE IMPLEMENTATION OF THE BASELINE SURVEY ON THE PREVALENCE OF *SALMONELLA* SPP. AND MRSA IN HERDS OF BREEDING PIGS

Reporting period:

— ..... to ..... For the *Salmonella* survey

— ..... to ..... For the MRSA survey

Statement on costs incurred in the survey and eligible for Community financial contribution:

Reference number of Commission Decision providing Community financial contribution:

.....

.....

Costs incurred related to functions at/by	Number of tests/swabs	Total costs of testing and swabs incurred during the reporting period (national currency)
Bacteriology for *Salmonella* spp.		
Serotyping *Salmonella* isolates		
Detection of MRSA		
Identifications of MRSA by PCR(s)		
Spa typing MRSA		
MLST typing MRSA		
Swabs for MRSA testing		

Declaration by the beneficiary

I certify that:

— the costs listed above are genuine and have been incurred in carrying out the tasks laid down in Decision 2008/55/EC and were essential for the proper performance of those tasks,

— all supporting documents for those costs are available for audit purposes,

— no other Community financial contribution has been requested for these surveys.

Date:

Person financially responsible:

Signature: