SCIENTIFIC OPINION

Scientific Opinion on monitoring procedures at slaughterhouses for bovines[1]

EFSA Panel on Animal Health and Welfare (AHAW)[2,3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

This scientific opinion proposes toolboxes of welfare indicators for developing monitoring procedures at slaughterhouses for bovines stunned with penetrative captive bolt or slaughtered without stunning. In particular, the opinion proposes welfare indicators together with their corresponding outcomes of consciousness, unconsciousness or death. In the case of slaughter with captive bolt stunning, the opinion proposes a toolbox of indicators and the outcomes to be used to assess consciousness in bovine animals at three key stages of monitoring: (a) after stunning and during shackling and hoisting; (b) during neck cutting or sticking; and (c) during bleeding. For slaughter of bovines without stunning, a set of indicators and outcomes are proposed in another toolbox to be used for (a) assessing unconsciousness, before releasing bovines from restraint; and (b) confirming death before carcass dressing begins. Various activities—including a systematic literature review, an online survey and stakeholders' and hearing experts' meetings—were conducted to gather information about the specificity, sensitivity and feasibility of the indicators that can be included in the toolboxes. The frequency of checking differs according to the role of each person responsible for ensuring animal welfare. Personnel performing stunning, shackling, hoisting and/or bleeding will have to check all the animals and confirm that they are not conscious following stunning or before release from the restraint. For the animal welfare officer, who has the overall responsibility for animal welfare, a mathematical model for the sampling protocols is proposed, giving some allowance to set the sample size of animals that he/she needs to check at a given throughput rate (total number of animals slaughtered in the slaughterhouse) and tolerance level (number of potential failures). Finally, different risk factors and scenarios are proposed to define a 'normal' or a 'reinforced' monitoring protocol, according to the needs of the slaughterhouse.

© European Food Safety Authority, 2013

KEY WORDS

stunning, slaughter, consciousness, death, welfare indicators, monitoring procedures

---

[1] On request from the European Commission, Question No EFSA-Q-2012-00840, adopted on 23 October 2013.

[2] Panel members: Edit Authie, Charlotte Berg, Anette Bøtner, Howard Browman, Ilaria Capua, Aline De Koeijer, Klaus Depner, Mariano Domingo, Sandra Edwards, Christine Fourichon, Frank Koenen, Simon More, Mohan Raj, Liisa Shivonen, Hans Spoolder, Jan Arend Stegeman, Hans-Hermann Thulke, Antonio Velarde, Ivar Vågsholm, Preben Willeberg and Stéphan Zientara. Correspondence: @efsa.europa.eu

[3] Acknowledgement: The Panel wishes to thank the members of the Working Group: Charlotte Berg, Mohan Raj, Hans-Hermann Thulke, Hans Spoolder, Antonio Velarde for the preparatory work on this scientific opinion and the hearing experts: Bosse Algers, Haluk Anil, Antonio Benlloch, Terence Cassidy, Rebeca Garcia, Marien Gerritzen, Karen von Holleben, Charlie Mason, Luc Mirabito, Elisiv Tolo and Cees Vermeeren, and EFSA staff: Denise Candiani, Chiara Fabris and Maria Ferrara for the support provided to this scientific opinion.

Suggested citation: EFSA AHAW Panel (EFSA Panel on Animal Health and Welfare), 2013. Scientific Opinion on monitoring procedures at slaughterhouses for bovines. EFSA Journal 2013;11(12):3460, 65 pp. doi:10.2903/j.efsa.2013.3460

Available online: www.efsa.europa.eu/efsajournal

© European Food Safety Authority, 2013

SUMMARY

Following a request from the European Commission (EC), the Panel on Animal Health and Welfare was asked to deliver scientific opinions on monitoring procedures at slaughterhouses for different animal species, stunning methods and slaughter without stunning. In particular, the opinions will (i) provide indicators assessing signs of (a) consciousness, in the case of slaughter with stunning, and (b) unconsciousness and (c) death of the animals, in the case of slaughter without stunning, which have been selected based on their performance (i.e. the sensitivity, specificity and feasibility of the indicator); (ii) indicate the most common risk factors and their welfare consequences to determine the circumstances of the monitoring procedures; and (iii) provide examples of sampling protocols, based on different possible scenarios.

The current opinion deals with the assessment of consciousness in bovines after stunning with penetrative captive bolt and the assessment of unconsciousness and death in bovines during slaughter without stunning. The Working Group agreed that although the tradition is to look for outcomes of unconsciousness in bovines following stunning, the risk of poor welfare can be detected better if bovine welfare monitoring was focused on detecting consciousness, i.e. ineffective stunning or recovery of consciousness. Therefore the indicators were phrased neutrally (e.g. corneal reflex) and the outcomes were phrased either suggesting unconsciousness (e.g. absence of corneal reflex) or suggesting consciousness (e.g. presence of corneal reflex). This approach is used commonly in animal health studies (e.g. testing for the presence of a disease) but very new to animal welfare monitoring in slaughterhouses. A toolbox of selected indicators is proposed to check for signs of consciousness in bovines after penetrative captive bolt stunning; a different toolbox of indicators is proposed for confirming unconsciousness as well as death of the animals following slaughter without stunning. Various activities (two stakeholder consultations, a systematic literature review, an online survey addressed to experts involved with monitoring welfare at slaughter) were carried out in order to obtain information on the sensitivity, specificity and feasibility of the indicators. Based on such information, the most appropriate indicators were selected and a toolbox of indicators to be used in monitoring procedures was proposed. The use of animal-based indicators is similar to the use of a diagnostic or statistical ‘test’ with either a positive or negative outcome. In the case of stunning of animals, the major interest is to detect the undesired outcome, namely the presence of consciousness in animals. The toolbox proposes respective indicators and their outcomes. In the case of slaughter without stunning, the interest is to detect whether the animals become unconscious and to detect when the animal dies. However, the indicators applied for this task also have to correctly detect animals as conscious or alive. In the case of slaughter without stunning, therefore, the toolbox proposes respective indicators and their outcomes.

Each of the toolboxes provides a set of recommended indicators and another set of additional indicators. The people responsible for monitoring have to choose the most appropriate set of indicators (at least two indicators) from these toolboxes according to their expertise and the available infrastructure in the slaughterhouse.

Toolboxes for slaughter with prior stunning:

After stunning of the animals prior to slaughter the indicators should be repeatedly checked to detect signs of consciousness through the three key stages of monitoring during the slaughter process: after stunning (between the end of stunning and shackling), during neck cutting or sticking and during bleeding.

The recommended indicators in Toolbox 1 (for monitoring after stunning) are posture, breathing, tonic seizure and the corneal reflex. Additionally, the indicators muscle tone, eye movements and vocalisation may be used.

In the case of Toolbox 2 (for monitoring at neck cutting or sticking), the recommended indicators are body movements, muscle tone and breathing. Additionally, eye movements, corneal or palpebral reflex and spontaneous blinking may be used.

For Toolbox 3 (for monitoring during bleeding) the recommended indicators are muscle tone, breathing and spontaneous blinking. There are no additional indicators.

Toolboxes for slaughter without stunning:

In the case of slaughter without stunning, all bovines should be checked until they become unconscious, before being released from the restraint; and death should be confirmed before starting carcass dressing. Moreover, consciousness or life in checked animals should be correctly identified. On this basis, the indicators were selected for the toolboxes. In addition, experts felt that carotid artery ballooning (carotid artery aneurysms and occlusion), which is a major risk factor prolonging the time to unconsciousness or recovery of consciousness in bovines subjected to slaughter without stunning, should be included in these toolboxes such that people responsible for animal welfare monitoring will be able to identify the risk and take the necessary action.

The recommended indicators in Toolbox 4 (for monitoring unconsciousness before release from the restraint) are breathing and muscle tone. Additionally, posture, corneal or palpebral reflex may be used.

Toolbox 5 (for monitoring death before carcass dressing begins) recommends bleeding, muscle tone and pupil size as indicators to check for death.

The personnel performing pre-slaughter handling, stunning, shackling, hoisting and/or bleeding will have to check all the animals to rule out the presence of consciousness following captive bolt stunning or to confirm unconsciousness and death during slaughter without stunning. The person in charge of monitoring the overall animal welfare at slaughter (i.e. the animal welfare officer) has to check a certain sample of slaughtered animals for approval. A mathematical model is proposed which can be used to calculate the sample size that he/she needs to check at a given throughput rate (total number of animals slaughtered in the slaughter plant) and tolerance level (number of potential failures—animals that are conscious after captive bolt stunning. Finally, different risk factors and scenarios are proposed to define, in addition to a ‘normal’ sampling procedure, a ‘reinforced’ protocol to be used if required by the particular circumstances and needs of the slaughterhouse.

TABLE OF CONTENTS

BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

Article 16 of Council Regulation (EC) No 1099/2009[4] on the protection of animals at the time of killing[5] requires slaughterhouse operators to put in place and implement monitoring procedures in order to check that their stunning processes deliver the expected results in a reliable way.

Article 16 refers to Article 5 which requires operators to carry out regular checks to ensure that animals do not present any signs of consciousness or sensibility in the period between the end of the stunning process and death.

Those checks shall be carried out on a sufficiently representative sample of animals and their frequency shall be established taking into account the outcomes of previous checks and any factors which may affect the efficiency of the stunning process.

Article 5 also requires operators, when animals are slaughtered without stunning, to carry out systematic checks to ensure that the animals do not present any signs of consciousness or sensibility before being released from restraint and do not present any sign of life before undergoing dressing or scalding.

According to Article 16(2), a monitoring procedure shall include in particular the following:

(a) indicators designed to detect signs of unconsciousness and consciousness or sensibility in the animals (before death or release from restraint, in case of slaughter without stunning, = indicators A); or indicators designed to detect the absence of signs of life in the animals slaughtered without stunning (before undergoing dressing or scalding = indicators B);

(b) criteria for determining whether the results shown by the indicators previously mentioned are satisfactory;

(c) the circumstances and/or the time when the monitoring must take place

(d) the number of animals in each sample to be checked during the monitoring.

Furthermore, Article 16 (4) specifies that: "*The frequency of the checks shall take into account the main risk factors, such as changes regarding the types or the size of animals slaughtered or personnel working patterns and shall be established so as to ensure results with a high level of confidence*."

The Commission plans to establish EU guidelines concerning monitoring procedures at slaughterhouses.

The purpose of the Commission is to provide a sort of "toolbox" for establishing monitoring procedures so that slaughterhouse operators can use scientifically based procedures which will provide them proper information on their stunning processes. The guidelines will also be used by the competent authorities in order to check that slaughterhouse operators are not using unreliable monitoring procedures.

In order to prepare these guidelines, a sound basis for checks on stunning as laid down in Articles 5 and 16 of the above-mentioned regulation is needed.

4 http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri = OJ:L:2009:303:0001:0030:EN:PDF

5 OJ L 303, 18.11.2009, p. 1.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

The Commission therefore considers it opportune to request the EFSA to provide an independent view on the indicators and elements for putting in place monitoring procedures at slaughterhouses for the following methods and scope, in light of the most recent scientific developments.

- The scope of this request includes the following groups of methods/species6:

  (1) penetrative captive bolt for bovine animals,

  (2) head-only electrical stunning for pigs,

  (3) head-only electrical stunning for sheep and goats,

  (4) electrical waterbath for poultry (chickens and turkeys),

  (5) carbon dioxide at high concentration for pigs,

  (6) all authorised gas methods to slaughter chickens and turkeys (carbon dioxide in two phases, carbon dioxide associated with inert gases and inert gases alone).

  (7) Slaughter without stunning for bovine animals,

  (8) Slaughter without stunning for sheep and goats,

  (9) Slaughter without stunning for chickens and turkeys.

- For each group the EFSA, based on the relevant scientific basis and on indicators' performances, will provide indicators A (loss of consciousness or sensibility for all groups) or indicators B (absence of signs of life for groups 7 to 9 only) as well as the other elements of the monitoring procedure (criteria for satisfactory results in terms of animal welfare, circumstances and sampling procedure, including minimum sampling and frequency) (sampling procedures are needed only for groups 1 to 6 since checks must be systematic for groups 7 to 9).

- For that purpose, the EFSA will take into account that:

  – Indicators should be able to detect, with high level of confidence, unsatisfactory stunning/slaughtering practices within the sample observed. Hence, the EFSA should specify the criteria for selecting indicators, based on the level of sensitivity and specificity for each indicator.

  – At least two indicators are required for each process but more could be recommended.

  – Indicators will be used at slaughterhouses, which imply human (work safety, accessibility), physical (line speed, difficulties to observation, etc.) and economic (time, costs) constraints. Hence, the EFSA could indicate the possible limitations related to the measurement of each indicator.

  – Circumstances to determine the monitoring procedure have to address the risk factors most commonly associated with each group methods/species (for example the penetrative captive bolt is likely to be more sensitive to the competence of the staff than a highly mechanised method). Hence, for each groups of methods/species, the EFSA should

[6] Wording used for the stunning methods refers to Annex I to Regulation (EC) No 1099/2009.

indicate the most common risk factors and their welfare consequences to determining the circumstances of the monitoring procedure (e.g. when the staff shifts if staff is an important risk factors).

– Monitoring procedures can be dynamic instruments and different indicators and sampling procedures could be used on the same slaughter line depending on the previous results and other risk factors. Hence, based on different possible scenarios, the EFSA should provide examples of different sampling protocols (like “new line/reinforced”, “regular”, “light”) and the minimum sampling needed for indicators ‘A’ (even when results appear to be fully satisfactory).

ASSESSMENT

1. Introduction

1.1. General introduction

According to Council Regulation (EC) No 1099/2009, on the protection of the animals at the time of neck cutting, animals must be rendered unconscious and insensible by the stunning method and they must remain so until death occurs through bleeding. One way of achieving this animal welfare requirement would be to monitor the state of consciousness/sensibility and unconsciousness/insensibility in animals at three key stages: (1) immediately after stunning, (2) at the time of neck cutting or sticking and (3) during bleeding until death occurs.

Within the scope of this Regulation, it is the responsibility of the food business operator (FBO) to ensure that the welfare of the animals is not compromised from the time of their arrival until they are slaughtered.

The ‘personnel’ performing pre-slaughter handling, stunning, shackling, hoisting and/or bleeding (hereafter referred to as the ‘personnel’) must hold a certificate of competence, awarded after training and assessment by independent organisations, attesting that they have the knowledge and skills required to recognise the signs of both effective and ineffective stunning and, in the event of a failure, to re-stun the animal. In addition, the ‘personnel’ performing slaughter (neck cutting, i.e. severing the carotid arteries, or sticking, i.e. severing the brachiocephalic trunk) should also have a certificate of competence attesting that they are aware of the need, and have the skills required, not only to perform prompt and accurate slaughter but also to check for signs of recovery of consciousness and sensibility prior to neck cutting or sticking to ensure that every animal is unconscious at the time of neck cutting or sticking. The personnel should also be able to ascertain the possibility or potential for recovery of consciousness in animals during bleeding and take action, if necessary (e.g. use a back-up stunner).

Finally, the person in charge of the overall animal welfare at slaughter (i.e. the animal welfare officer) should be able to monitor the animals during the entire process, from stunning to bleeding, and ascertain that they do not show any signs of consciousness and sensibility and also that death occurs before further carcass dressing operations begin. Under laboratory conditions, the induction and maintenance of unconsciousness and insensibility following stunning can be ascertained by recording the brain activity using electroencephalogram (EEG) or electrocorticogram (ECoG). The effectiveness of stunning and the duration of unconsciousness induced by the stunning method can be recognised from the unique brain state and associated EEG manifestations. When stunning-induced EEG or ECoG changes are ambiguous, abolition of somatosensory or visual evoked potentials in the brain has been used to ascertain the brain responsiveness to these external stimuli. The effectiveness of stunning and neck cutting can also be recognised under field conditions from the characteristic changes in the behaviour of animals (e.g. loss of posture), physical signs (e.g. onset of seizures, cessation of breathing, fixed eye) and from the presence or absence of response to physiological reflexes (e.g. response to an external stimulus such as blinking response to touching the cornea (corneal reflex), response to pain stimulus such as nose prick or toe pinching). In the scientific literature, these physical signs and reflexes have been referred to as indicators of unconsciousness or consciousness and are used to monitor welfare at slaughter of animals (see, for example, EFSA, 2004).

At all of the key stages, monitoring is carried out to identify animals that are improperly stunned, and therefore attention is focused on the indicator of consciousness. Effectively stunned animals are expected to remain unconscious during key stages 2 and 3 until death occurs. It is thought that, for this monitoring system to be effective, it is important to define indicators (see sections 3.4 and 3.5 and the glossary), to identify the pathophysiological basis of the stunning method and its relevance or appropriateness to key stages of monitoring, and also to describe how the indicator may be manifested or can be used to recognise consciousness at a particular key stage of monitoring.

The slaughter of animals without prior stunning is regulated by Article 4 (4) of Regulation (EC) No 1099/2009. Slaughter without stunning induces gradual loss of consciousness, and consequently death, by depriving the brain of nutrients and oxygenated blood, leading to the onset of brain ischaemia. According to the Regulation, people performing slaughter without stunning are required to have a certificate of competence. The Regulation also stipulates that all ruminants must be mechanically restrained during slaughter and shall be released from the restraint only when unconsciousness has ensued. Carcass dressing shall begin after the onset of death. Therefore, it is important to define indicators that can be used to recognise unconsciousness and death following slaughter without stunning while simultaneously recognising as such any animal still conscious or alive.

1.2. Definitions

Consciousness is a state of awareness which requires the function of the brain stem and projections in the relevant cortical regions. Following everyday neurological practice (Zeman, 2001), consciousness is generally equated with the waking state and the abilities to perceive, interact and communicate with the environment and with others, which is referred to as sensibility. Consciousness is a matter of degree, and a range of conscious states extends from waking through sleep until unconsciousness is reached. For the purpose of this opinion, an animal is described as 'conscious' as long as a degree of consciousness is detected.

Unconsciousness is a state of unawareness (loss of consciousness) in which there is temporary or permanent damage to brain function and the individual is unable to perceive external stimuli (which is referred to as insensibility) and control its voluntary mobility and, therefore, respond to normal stimuli, including pain (EFSA, 2004).

In the Dialrel project (von Holleben, 2010), the definition of 'unconsciousness' was similar to that used by anaesthesiologists: "*Unconsciousness is a state of unawareness (loss of consciousness) in which there is temporary or permanent disruption to brain function. As a consequence the individual is unable to respond to normal stimuli, including pain*."

According to Regulation 1099/2009, the **sensibility** of an animal is essentially its ability to feel pain. In general, an animal can be presumed to be insensible when it does not show any reflexes or reactions to stimuli such as sound, odour, light or physical contact.

In the context of this scientific opinion, consciousness includes sensibility and unconsciousness includes insensibility.

Death is a physiological state of an animal, in which respiration and blood circulation have ceased as the respiratory and circulatory centres in the medulla oblongata are irreversibly inactive. Owing to the permanent absence of nutrients and oxygen in the brain, consciousness is irreversibly lost. In the context of application of stunning and stun/kill methods, the main clinical signs of death are absence of respiration (and no gagging), absence of pulse and dilated pupils (EFSA, 2004).

1.3. Physiology of penetrative captive bolt stunning

Captive bolt stunning induces immediate loss of consciousness and sensibility as the impact of the bolt on the skull results in brain concussion. The effects are prolonged by the structural damage to the brain caused by penetration of the bolt, which results in marked subarachnoid and intraventricular haemorrhages, especially adjacent to the entry wound and at the base of the brain. While the substantial damage sustained by vital centres in the caudal regions of the brain including the brain stem rapidly renders the animal unconscious, it is the major haemorrhages caused by rupture of the arteries supplying the brain that ensures a long-lasting unconsciousness, during which sticking procedures may be implemented, and eventual death. The neurophysiological basis of brain concussion and the consequences of structural damage to different regions of the brain are well documented in the scientific literature (see EFSA, 2004, for details).

Successful induction of brain concussion manifests as immediate collapse of the animal and onset of apnoea (absence of breathing), followed by the onset of a tonic seizure, which can be recognised from the occurrence of arched back and legs flexed under the body, and fixed eyes. The duration of the tonic seizure is influenced by several factors (e.g. category of bovine, type of captive bolt gun and ammunition), but usually lasts for seconds and is followed by loss of muscle tone, which can be recognised from drooping ears, relaxed jaw, protruding tongue and limp tail and legs, especially when the animals have been shackled and hoisted on to the overhead bleeding rail. Reflexes that would require brain control are also abolished. For example, the palpebral (elicited by touching eyelashes or inner or outer canthus of the eye), corneal (elicited by touching the cornea with a finger or paint brush) and pupillary (elicited by focusing a bright light on the pupil) reflexes and response to external stimuli including pain (e.g. nose prick) are also abolished during the period of unconsciousness.

Ineffective or unsuccessful captive bolt stunning in animals can occur for various reasons and, as a consequence, the animal may not suffer brain concussion and/or structural damage to the brain of the magnitude required to achieve unconsciousness, leading to different behavioural manifestations and retention of some reflexes. This situation can be recognised from the failure to collapse, the presence of breathing (including laboured breathing) and the absence of tonic seizure; in extreme cases, animals may also vocalise. Movements of the eyeball (including nystagmus) are also a sign of ineffective captive bolt stunning. The ineffectively stunned animal may collapse partially but retain some muscle tone and, as a consequence, attempt to regain posture, i.e. stand upright again. Ineffectively stunned animals and those recovering consciousness will show spontaneous blinking or positive eye reflexes (palpebral, corneal and pupillary) and vigorous kicking, especially of the hind legs. Head righting (attempt to raise head) after stunning and body arching during bleeding are also signs of consciousness.

Effectively stunned animals, i.e. unconscious cattle, are bled out by inserting a knife through their thoracic inlet and cutting the brachiocephalic trunk. In certain circumstances, neck cutting involving severance of the carotid arteries may be used. Prompt and accurate sticking of effectively stunned animals results in rapid death, and therefore animals do not show signs of recovery of consciousness at any key stages of monitoring. This means that, if stunning has been effective, and the duration of unconsciousness induced by the stunning method is longer than the sum of time between the end of stunning and sticking (stun-to-stick interval) plus the time it takes for animal to die through blood loss, the animal will remain unconscious until death occurs. In contrast, ineffective stunning or prolonged stun-to-stick interval and/or inappropriate/inadequate neck cutting or sticking will lead to animals showing signs of recovery of consciousness. In this regard, it is worth noting that aneurysm and occlusion of the carotid artery (commonly known as carotid artery ballooning) is common in bovines following neck cutting and has not been reported following thoracic sticking. Occlusion of the carotid arteries occurs within a few seconds of neck cutting and is known to prolong the time to onset of unconsciousness; thus, it is important that the signs of carotid artery ballooning are recognised at an early stage, and appropriate intervention undertaken to minimise the chances of poor welfare following slaughter without stunning.

1.4. Physiology of slaughter without stunning

Slaughter without stunning does not induce immediate loss of consciousness in animals. In other words, animals are gradually rendered unconscious by the severance of the carotid arteries as brain perfusion becomes insufficient to sustain normal function, eventually leading to death. The times to onset of unconsciousness and to death are highly variable between animals. This is because bleeding may not always be profuse or uninterrupted, for example as a result of incomplete severance of the carotid arteries (poor cut), physical obstruction to the blood flow from the cut ends, or the formation of aneurysms in the cut ends of the carotid arteries, leading to occlusion. All these factors will lead to poor welfare, and therefore continuous and systematic monitoring of all animals slaughtered without stunning is required.

In the conscious animal, the cerebral cortex integrates posture and movement. Collapse, which manifests when a freely standing animal falls to the ground, is the earliest indication of approaching unconsciousness after the neck cut (Blackmore, 1984; Grandin, 1994; Gregory et al., 2010), as it indicates that the cortex is no longer able to control postural stability (Muir, 2007). However, an animal that has collapsed after a dramatic loss of blood pressure may nevertheless have the capacity to regain consciousness as a result of the body's own counter-regulation mechanisms (von Holleben et al., 2010). Gregory et al. (2010) described the loss of posture of adult cattle after slaughter without stunning in upright restrained position as follows: "*When the cattle were released from the head restraint, most stepped backwards, stood for varying lengths of time, swayed or became unsteady and then either fell to one side and slid down the wall or their hind limbs buckled and they fell backwards followed by loss of support from the forelimbs. When down, some animals sat in sternal recumbency, but most fell into lateral recumbency or were leaning laterally. Loss of posture occurred on average at 19.5 seconds post cut (median 11 s, maximum 265 s)".*

Positive pupillary, palpebral and corneal reflexes, response to threatening movements and response to nose prick—which are reflexes controlled by cranial nerves—assist in ascertaining the magnitude of brain dysfunction. If all are negative, good outcomes of impaired midbrain or brain stem activity and unconsciousness can be inferred, provided the muscles and afferent and efferent nerves which execute the response are still capable of working and are not preoccupied with other stimuli (Gregory, 1998). Cognitive responses can be also assessed in order to evaluate cranial nerve responses. For example, reaction in animals to a threat stimulus, such as movement of a hand towards the eye (Limon et al., 2010) or clapping the hands, indicates consciousness.

When the function of the brain stem is sufficiently impaired as a result of the blood loss, respiration will cease and the heart will also cease functioning over time (Pallis, 1982a, b, c, d; Michiels, 2004; Rosen, 2004). The main clinical signs of death are permanent absence of respiration (and also absence of gagging) and absence of a pulse.

2. Materials and methods

2.1. Indicators and criteria for selection of the indicators

The mandate requests EFSA to select:

- **Indicators A**: designed to detect signs of consciousness in the animals after stunning or, in the case of slaughter without stunning, signs of unconsciousness (before release from restraint in the case of slaughter without stunning).

- **Indicators B**: designed to detect—in the animals slaughtered without stunning—signs of death before undergoing dressing or scalding.

For the sake of clarity and consistency, in this opinion, indicators of the state of consciousness and unconsciousness will be used instead of indicators A and indicators of the state of life and death will be used instead of indicators B, as shown in Table 1.

The Working Group agreed that, although it is traditional to look for outcomes of unconsciousness in animals following stunning, the risk of poor welfare is more likely to be detected if animal welfare monitoring is focused on detecting consciousness, i.e. ineffective stunning or recovery of consciousness. Therefore, the indicators were phrased neutrally (e.g. posture) and the outcomes were phrased either suggesting unconsciousness (e.g. immediate collapse) or suggesting consciousness (e.g. no collapse/attempts to regain posture). This approach is commonly used in animal health studies (e.g. testing for the presence of a disease) but very new to animal welfare monitoring in slaughterhouses.

Table 1: Correspondence between indicators suggested in the ToR of the mandate and indicators proposed in this scientific opinion

Species	Method	Key stage	Indicators		
			Indicators from mandate's ToRs	**Checking state of**	**Outcome related to animal welfare**
Bovine animals	Stunning with penetrative captive bolt	Key stage 1 = immediately after stunning until shackling	A	Consciousness and unconsciousness	Consciousness
		Key stage 2 = during neck cutting or chest sticking	A	Consciousness and unconsciousness	Consciousness
		Key stage 3 = during bleeding	A	Consciousness and unconsciousness	Consciousness
	Slaughter without stunning	Prior to release from restraint	A	Consciousness and unconsciousness	Consciousness
		Prior to dressing	B	Life and death	Life

The indicators investigated in this opinion were selected based on previous EFSA opinions (EFSA, 2004, 2006) and amended in Working Group discussion on the basis of feedback from (i) a stakeholder meeting, at which interested parties were asked to complete a questionnaire (referred to in this opinion as questionnaire 1), (ii) a systematic literature review, (iii) an online survey of experts involved in monitoring welfare at slaughter or neck cutting in the form of a questionnaire (questionnaire 2), (iv) public consultation on the scientific opinion on bovines and (v) a technical meeting of selected experts. Their suitability for inclusion in a monitoring system was determined during Working Group discussions on the basis of their sensitivity and specificity, and their feasibility for use at different key stages of the slaughter process.

2.1.1. Feasibility

The feasibility of an indicator is considered in relation to physical aspects of its assessment. These include the position of the animal relative to the assessor, the assessor's access to the animal and the line speed. Feasibility for the purpose of this opinion does not include economic aspects. It is very likely that the feasibility of assessing an indicator is influenced by the key stage of the slaughter process, i.e. after stunning, at sticking/neck cutting and during bleeding animals can be in different positions and proximity relative to the assessor, which may affect how easily the indicator can be used.

2.1.2. Sensitivity and specificity

The use of animal-based indicators is similar to the use of a diagnostic or statistical test with either a positive or negative outcome. The performance of a test (i.e. the indicator) is usually described by its sensitivity and specificity. The estimation of sensitivity and specificity requires a definition of what can be considered a positive or negative outcome of checking for an indicator. Definitions of the sensitivity and specificity of indicators differ depending on whether they are used in situations where animals are slaughtered with or without stunning.

2.1.2.1. Sensitivity and specificity during slaughter with stunning

When monitoring the effectiveness of the stunning, in order to safeguard animal welfare, it is of major interest to detect those animals that are not properly stunned or recover consciousness after stunning. A positive outcome of the checked indicator is that based on which the animal is considered conscious. A negative test outcome of the indicator is that based on which the animal is considered not conscious (i.e. animal is considered unconscious).

Sensitivity is thus calculated as the number of truly conscious animals considered conscious based on the outcome of the indicator (A in Table 2) divided by the number of all conscious animals (A + C), multiplied by 100 (in short, sensitivity is the percentage of truly conscious animals that the indicator tests as conscious).

Specificity is calculated as the percentage of truly unconscious animals (B + D) that the indicator does not test conscious (D).

Table 2: Sensitivity and specificity of indicators during slaughter with stunning

Slaughter with stunning		Truth: the animal is conscious?	
		Yes	No
Is the animal considered conscious, based on the outcome of the indicator?	Yes	**A**	B
	No	C	**D**

An indicator for slaughter with prior stunning is considered to be 100 % sensitive if it detects all the conscious animals as conscious; an indicator is considered to be 100 % specific if it detects all the unconscious animals as unconscious.

2.1.2.2. Sensitivity and specificity during slaughter without stunning

In contrast, during slaughter without stunning, all the animals are live and conscious when neck cutting is performed and the purpose of the cut is to induce unconsciousness followed by death. Therefore, it is of major interest to detect unconsciousness and death in all animals. The use of indicators for detecting unconsciousness or death is a test with positive or negative outcome, where the positive outcome causes the animal to be considered as conscious or alive, and the negative outcome is the confirmation of unconsciousness or death, respectively.

In this case, sensitivity is calculated as the number of conscious or alive animals considered conscious or alive based on the outcome of the indicator (E in Table 3) divided by the number of conscious or alive animals (E + G), respectively, multiplied by 100 (in short: the percentage of animals truly still conscious or alive that the indicator tests conscious or alive).

Specificity is calculated as the percentage of unconscious or dead animals (F+H) that the indicator does test as unconscious or dead (H), respectively.

An indicator for slaughter without stunning is considered to be 100% sensitive if it detects all animals still conscious or alive as conscious or alive animals. An indicator is considered to be 100% specific if it detects unconsciousness or death in animals, when animals truly became unconscious or dead, respectively.

Table 3: Sensitivity and specificity of indicators during slaughter without stunning

Slaughter without stunning		Truth: the animal is still conscious (or alive):	
		Yes	No
Is the animal considered conscious (or alive) based on the outcome of the indicator?	Yes	**E**	F
	No	G	**H**

2.2. Establishing the ability of the indicators to detect welfare problems at slaughter

2.2.1. Stakeholder meeting and questionnaire 1

A stakeholder meeting was held on 30 January 2013 in order to inform all interested parties about this mandate. The meeting was opened to participants from all EU Member States representing research groups, FBOs licensed to own premises to slaughter animals, animal welfare officers employed by the FBO, auditing companies, the European Commission, Member State Competent Authorities, members of EFSA's Stakeholders Consultative Platform and non-governmental organisations (NGOs) with proven experience in the field of humane slaughter. The meeting was an opportunity for the experts to exchange experience and information on the animal-based indicators most commonly used to check unconsciousness and death in bovines, during slaughter with or without stunning. More than 100 experts or persons claiming to be experts associated with the slaughter of animals (not restricted to bovines) participated in the meeting. Traditionally, animal welfare monitoring in slaughterhouses involves checking for unconsciousness or death, following the application of a stunning method. Therefore, a questionnaire on the use of animal-based indicators to check for the state of consciousness and unconsciousness or life and death at slaughter was distributed to all participants. The questionnaire asked about (i) the indicators that are mostly used and their use in combination; (ii) the timing of the assessment of unconsciousness and death based on such indicators; (iii) problems encountered during the assessment (feasibility of the indicators); and (iv) the respondent's opinion of the reliability of the indicators. The participants were also asked to suggest names of experts with practical knowledge in the field of slaughter to be contacted for the subsequent online survey (section 2.2.3).

2.2.2. Systematic literature review

A systematic literature review was conducted in order to summarise the currently available data describing the sensitivity and specificity of indicators checking the state of consciousness and unconsciousness or life and death for all stun-kill methods and species combinations (O'Connor et al, in press). Traditional animal welfare monitoring in slaughterhouses involves checking for outcomes of unconsciousness following the application of a stunning method. Therefore, in order to obtain information on sensitivity and specificity, the systematic review was conducted on studies in which outcomes of unconsciousness and outcomes of death were measured using electroencephalogram (EEG). In such studies, the indicators of interest (e.g. absence of corneal reflex, absence of breathing, loss of posture) were tested against the results of EEG (e.g. a stunned animal does not show a corneal reflex and its unconsciousness is confirmed by EEG measurement).

2.2.3. Questionnaire 2 (online survey)

In addition, an online survey was launched using a questionnaire to gather a subjective opinion from experts with knowledge and experience in stunning and slaughtering of animals. The conduct of the survey was outsourced to an external communication company and its final technical report can be found on EFSA's website (Sellke, in press). The survey was structured on the basis of the results from the questionnaire distributed at the stakeholder meeting held on 30 January 2013 and was addressed to approximately 160 participants. In order to avoid confusion, the assessments of feasibility, sensitivity and specificity of the indicators were presented in separate sections of the questionnaire. The EFSA Panel on Animal Health and Welfare agreed that, although it is traditional to look for outcomes of unconsciousness in animals following stunning, the risk of poor welfare is better detected if animal welfare monitoring is focused on detecting consciousness, i.e. ineffective stunning or recovery of consciousness. Therefore, the selected indicators were phrased neutrally (e.g. posture) and the outcomes were phrased positively, suggesting unconsciousness (e.g. immediate collapse), or negatively, suggesting consciousness (e.g. no collapse/attempts to regain posture). This approach is commonly used in animal health studies (e.g. testing for the presence of a disease) but very new to animal welfare monitoring in slaughterhouses.

Regarding feasibility, for each species and method, questions were asked on how easily the indicators are applied and checked at each key stage of the stunning and slaughter process and of the slaughter process without stunning. For each key stage the feasibility ratings were computed into a feasibility score across all respondents that weighed the proportion of ratings easy against the proportion of ratings difficult as presented in the equation below

Feasibility score = (No of 'easy' respondents – No of 'difficult' respondents)/Total No of respondents

For example, in the case of data distribution of easy = 3, normal = 6 and difficult = 1, the score would be +0.2, i.e. (3 – 1)/10.

The resulting score was between +1 and –1 and covers the median rating as well as the tendency across all ratings, thus providing an overview of the distribution of the data and associated variability.

In addition, the survey asked respondents to assess the sensitivity and specificity of the indicators. This information was elicited by asking respondents to estimate, for each indicator, the proportion of truly conscious and proportion of truly unconscious animals that would be considered conscious, based on the outcome of the indicator (i.e. A and B in Table 2). Sensitivity and specificity were estimated across all respondents using either the direct or weighted average of individual data values. The weights are provided by the uncertainty rating assigned by each respondent to every answer, which ranged between 1 and 3 (1 = 'not sure', 2 = 'rather sure', 3 = 'very sure'). A "don't know" option was also given. Prior to calculations, the data were closely examined for consistency and corrected according to the following rules: answers associated with the uncertainty rating 'don't know' were excluded (e.g. 18/388 for captive bolt stunning); if the uncertainty rating was omitted, answers were re-set to the lowest uncertainty weight (i.e. 1 = 'not sure'; 10/370); if a respondent's answers to all or the priming sequence of 'not show/not respond to' (i.e. 'breathing', 'nose prick') questions reversed the logic (i.e. "5 % of truly unconscious animals will not show eye movements") and the corresponding question was rather consistently answered by other respondents (in this case 19/20 respondents gave a rating above 80 %), then the values in the data record were reversed as '100 % minus rating' (10/370). Ratings were not reversed if variability across the respondents was too large for particular indicators to conclude logical inconsistency. Particularly for bovine data, two respondents were excluded as outliers as their answers indicated a misinterpretation of the questionnaire (22/370). Moreover, it was decided to exclude the answers of one respondent on truly conscious animals as these were logically inconsistent and copied in detail the part on unconscious animals including uncertainty rating values (e.g. data storage error; 6/348). In total 342 individual ratings were evaluated for captive bolt stunning.

2.2.4. Working Group discussions

The outcomes of all previous activities were assessed and discussed within the Working Group of experts developing this scientific opinion. In addition, a technical meeting with a group of external experts (five academics, two from NGOs, one representative from the poultry industry, one representative from the red meat industry and two representatives from the EC) was held on 3 September 2013. During the meeting, the results obtained during the preceding activities of the Working Group were discussed, with the aim of advising the Working Group on the content of the toolboxes for captive bolt stunning and for slaughter without stunning. The experts invited to this meeting had previous access to the draft opinion, including the toolbox of indicators, and were asked to give their comments. During the meeting various presentations were given to stimulate discussion. A public consultation on the draft scientific opinion was also held during August-September 2013 (EFSA AHAW Panel, in press).

2.3. Developing the sampling protocol

In order to develop a monitoring procedure for slaughter with stunning, the mandate from the Commission requests EFSA to estimate the optimal frequency with which animals should be checked for signs of consciousness following stunning. This sampling frequency should take into account risk factors associated with the stunning procedure. To calculate the optimal sampling fraction (or sampling frequency), at least two components need to be quantified: first, the highest proportion of insufficiently stunned animals that may be considered acceptable; and, second, the effects of the risk factors (individually or in combination) on the frequency of ineffective stunning.

Both components are problematic. Regarding the level of acceptability, the legislation specifies that no animals should show signs of consciousness following stunning. All animals should be stunned properly, and therefore the threshold level for the acceptability of ineffective stunning is zero. The second component requires a large number of data on the interactive effects of risk factors on stunning effectiveness, given a wide range of circumstances under which animals are stunned in European abattoirs. These data are not available.

However, it is possible to model the relationship between the fraction of slaughtered animals sampled and the minimum proportion of ineffectively stunned animals that will be detectable using a certain sampling protocol. Understanding this relationship allows the risk manager (and others concerned) to relate the economic and other costs associated with a particular sample size to the benefits associated with improved detection levels (i.e. improved animal welfare).

2.3.1. The statistical background of the model

The relationship can be modelled using existing approaches for process monitoring (e.g. continuous quality assurance regarding threshold failure rate in computer chip production). Although the statistical relationship is identical to those applied in planning disease surveillance, the related terminology (e.g. design prevalence) was considered less appropriate for addressing the issue of mis-stunned animals and therefore this text adheres to the terminology of failure management. For the statistical model, we used the following parameters:

1. Threshold failure rate for proportion of mis-stunned animals. This specifies the minimum proportion of animals that are ineffectively stunned which will still be detected by the sampling protocol.

2. Sensitivity of the indicators. As defined previously, this is the percentage of truly conscious animals detected as conscious by the indicator.

3. Slaughter population. This is the total number of animals slaughtered under the same circumstances as determined by risk factors (see Table 10). Note that the slaughter population is independent of the line speed, and can cover a period of minutes, hours or even days.

4. Sampling fraction. This is the proportion of the slaughter population which is assessed in the sampling protocol.

5. Accuracy of the sampling protocol. This is the percentage of situations in which the sampling protocol was applied and served its purpose, i.e. raising an alarm if the number of ineffectively stunned animals was higher than the prescribed threshold failure rate would allow.

Please note that for the captive bolt stunning situation, specificity is not considered for the purposes of this model, as the specificity of an indicator is not related to the risks associated with reduced welfare.[7]

Given these parameters, the details of the monitoring protocol can be calculated from Equation 1 (Cannon, 2001).

[7] It should be noted that an indicator of low specificity, although not representing an animal welfare issue, definitely represents an issue from a food business operator (FBO) perspective. An indicator with low specificity would more often misclassify unconscious animals as conscious. Obviously, this represents a problem from a FBO perspective as an unnecessary corrective action must be taken, entailing a waste of money and time.

$$SF = \frac{n}{SP} \cong \frac{\left(1-(1-A)^{1/(SP\cdot FR)}\right)\cdot(SP-0.5(A(SP\cdot FR)-1)}{ISe}$$

Where:

- A = requested accuracy of the sampling protocol
- FR = standard threshold failure rate
- ISe = indicator sensitivity
- *n* = number of animals tested
- SF = sample size or sampling fraction
- SP = slaughter population

The objective was to use Equation 1 to estimate the threshold FR associated with a given SF. However, Equation 1 cannot be solved for the FR in an algebraic way. For this reason, it was necessary to solve the equation numerically. For the purpose, the R[8] function ‘uniroot’ was used.

Solving Equation 1 numerically, it was then possible to determine the minimum detectable FR associated with each SF value. The results could then be plotted in a diagram (see Figure 1). Once the relationship is formalised, it is also possible to read the results the other way round, i.e. to estimate the minimum SF needed to detect a given FR, with a given accuracy, accounting for the indicator sensitivity and the slaughter population.

[8] R Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

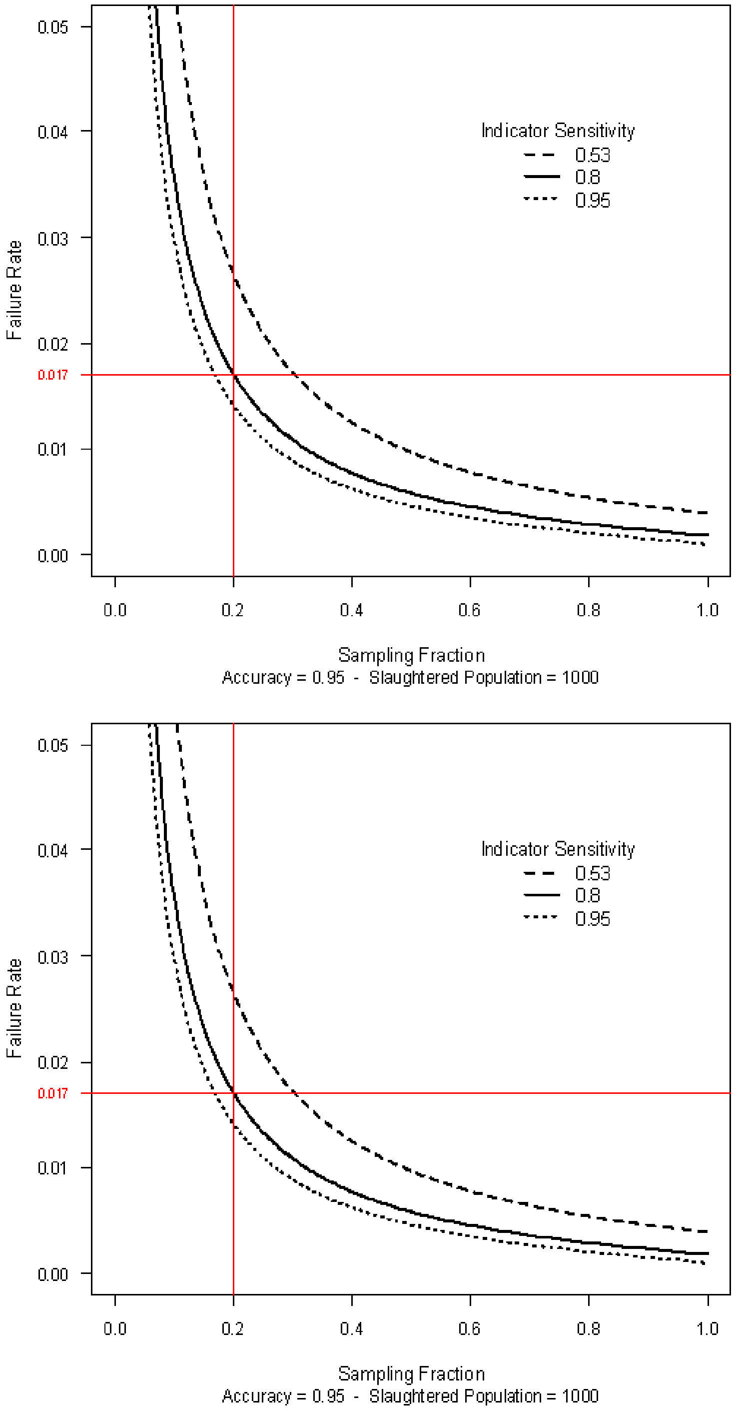


Figure 1: Example graph of the relationship between the parameters defining a sampling protocol (SF and detectable threshold FR for fixed values of accuracy (here 95 %) and slaughter population (here 1 000 animals) and various scenarios for indicator sensitivity)

In Figure 1, a slaughter population of 1 000 animals and a required accuracy of 95 % are assumed. The red horizontal and vertical lines on the diagram form the basis of the following illustration: using an indicator with a sensitivity of 80 % (solid line), a sampling fraction of 20 % (i.e. sample size of 200 animals from a slaughter population of 1 000 animals) will be able to detect, with 95 % accuracy, a threshold failure rate of 2 % (i.e. more than 20 conscious animals out of 1 000 animals slaughtered in this example) or greater. The dotted lines illustrate how this relationship changes with indicators of varying sensitivity.

Different scenarios were considered assuming alternative model parameters for the specification of the sampling protocol. In detail, the following scenarios were considered:

- accuracy: 0.90, 0.95, 0.99
- slaughter population: 100, 1 000, 10 000
- test sensitivity: 0.5, 0.75, 1

In order to compare the impact of these three parameters on the relationship between the threshold FR and the SF, the other two of them were set at fixed values. Then combinations of FR and SF were evaluated, to identify those that would trigger an alarm with the required accuracy, and those that would not. These critical combinations constitute the line graph exactly representing the desired accuracy level, e.g. in Figure 1. All 3 × 3 combinations were explored.

2.3.2. The resulting model for the sampling protocol

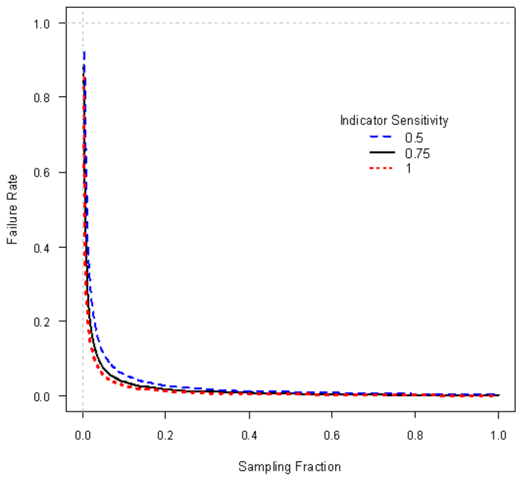
The results of the statistical modelling are summarised in Figure 2.

Using the five parameters of the model presented in Equation 1, it is possible to calculate each of them if the other four are specified. To illustrate the influence of the different parameters, the full range of FR[9] and SF values were combined with (a) the sensitivity of the indicator, (b) the slaughter population of the slaughterhouse[10] and (c) the desired accuracy of the sampling protocol[11] whilst keeping the other two parameters constant in each case. The impacts of different indicator sensitivity, slaughter population and accuracy values are presented in Figure 2a, b and c.

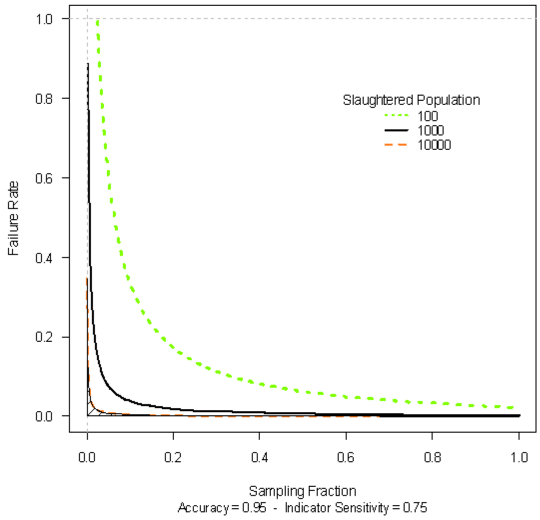
[9] Proportion of mis-stunned animals (see Section 2.3.1).

[10] The total number of animals being stunned during a given period according to the type of the slaughterhouse and the species slaughtered (see Section 2.3.1).

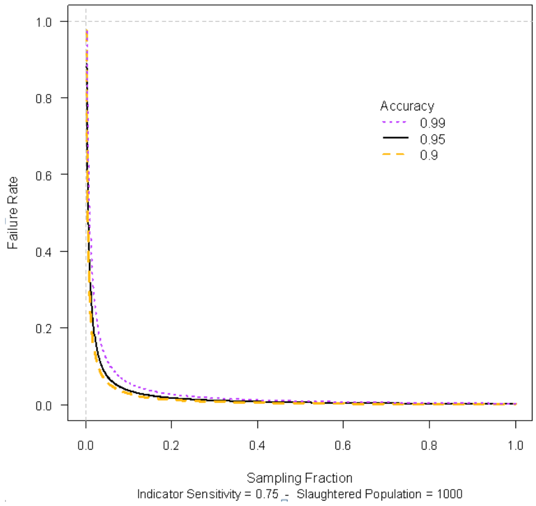
[11] Percentage of situations in which the sampling protocol was applied and served its purpose, i.e. raising an alarm if there were more ineffectively stunned animals than the prescribed failure rate would allow (see Section 2.3.1).



(a) The effect of SF on threshold FR for three levels of indicator sensitivity (0.5, 0,75 and 1), given a slaughter population of 1 000 animals and an accuracy of 0.95.



(b) The effect of SF on threshold FR for three levels of slaughter population (100, 1 000 and 10 000), given an accuracy of 0.95 and indicator sensitivity of 0.75.



(c) The effect of SF on threshold FR for three levels of accuracy (0.9, 0.95 and 0.99), given a slaughter population of 1 000 animals and indicator sensitivity of 0.75.

Figure 2: Effect of SF on threshold FR for three levels of indicator sensitivity (a), slaughter population (b) and accuracy (c), given a slaughter population of 1 000 animals (a, c), an accuracy of 0.95 (a, b) and indicator sensitivity of 0.75 (b, c)

Each *x*–*y*-coordinate in the diagrams represents one possible sampling protocol.

Those sampling protocols that fall below the line describing that combination of parameters will not be able to meet the purpose of detecting if FR is exceeded; those protocols above the line will meet the required purpose and raise an alarm.

Table 4a, b and c show numerical examples of FRs for three levels of indicator sensitivity, SF and sampling protocol accuracy.

Table 4: Effect of SF on threshold FR for three levels of (a) indicator sensitivity, given a slaughter population of 1 000 animals and accuracy of 0.95; (b) slaughter population, given an accuracy of 0.95 and indicator sensitivity of 0.75; and (c) accuracy, given a slaughter population of 1 000 animals and indicator sensitivity of 0.75

(a) Effect of SF on threshold FR for three levels of indicator sensitivity (0.5, 0,75 and 1), given a slaughter population of 1 000 animals and accuracy of 0.95

Sampling fraction	**Threshold failure rate**		
	Indicator sensitivity = 0.5	**Indicator sensitivity = 0.75**	**Indicator sensitivity = 1**
0.1	0.058	0.038	0.028
0.2	0.028	0.018	0.013
0.3	0.018	0.012	0.008
0.4	0.013	0.008	0.006
0.5	0.01	0.006	0.004
0.6	0.008	0.005	0.003
0.7	0.007	0.004	0.002
0.8	0.006	0.003	0.002
0.9	0.005	0.003	0.001
1	0.004	0.002	NA

(b) Effect of SF on threshold FR for three levels of slaughter population (100, 1 000 and 10 000 animals), given an accuracy of 0.95 and indicator sensitivity of 0.75.

Sampling fraction	**Threshold failure rate**		
	***n* = 100**	***n* = 1 000**	***n* = 10 000**
0.1	0.34	0.04	0
0.2	0.17	0.02	0
0.3	0.11	0.01	0
0.4	0.08	0.01	0
0.5	0.06	0.01	0
0.6	0.05	0.01	0
0.7	0.04	0	0
0.8	0.03	0	0
0.9	0.03	0	0
1	0.02	0	0

(c) Effect of SF on threshold FR for three levels of accuracy (0.9, 0.95 and 0.99), given a slaughter population of 1 000 animals and indicator sensitivity of 0.75.

Sampling fraction	**Threshold failure rate**		
	Accuracy = 0.9	**Accuracy = 0.95**	**Accuracy = 0.99**
0.1	0.029	0.038	0.058
0.2	0.014	0.018	0.028
0.3	0.009	0.012	0.018
0.4	0.006	0.008	0.013
0.5	0.005	0.006	0.01

0.6	0.004	0.005	0.008
0.7	0.003	0.004	0.006
0.8	0.003	0.003	0.005
0.9	0.002	0.003	0.004
1	0.002	0.002	0.003

3. Results

3.1. Results from stakeholder meeting

From the stakeholder meeting held on 30 January 2013, about 60 completed questionnaires were collected. Most of the experts provided information for more than one species and method: the total number of answers and the most used signs of unconsciousness and death in bovines are reported in Table 5.

Table 5: Total number of answers and the outcomes of unconsciousness and death of indicators most used for bovines as collected through questionnaire1 of the stakeholder meeting

Species/method	Total No of answers	Outcome of unconsciousness of most used indicators[12]	Outcome of death of most used indicators[13]
Bovines—captive bolt stunning	36	Immediate collapse No corneal reflex Immediate and sustained absence of rhythmic breathing	
Bovines—slaughter without stunning	12	No attempts to raise the head No righting reflex (i.e. attempts to regain posture) No corneal reflex	Absence of breathing Permanent collapse of the animal Dilated pupils

Experts responded that they observe the outcomes of the indicators between 10 and 30 seconds after stunning or after neck cutting. The main problem encountered in checking most of the indicators is the access to the animal. Another common problem is the difficulty of evaluating the indicators on different animal categories, for instance bulls versus dairy cows. Several indicators are normally used by the experts to assess the state of unconsciousness and death in animals. However, there was no harmonised list of indicators, either species or method specific, or scientific rationale.

3.2. Results from systematic literature review

The comprehensive literature search identified 22 studies, but no study explicitly reported the sensitivity and specificity of the indicators used to check the state of consciousness and unconsciousness. In two studies of captive bolt stunning in cattle, 100 % of unconscious animals tested as unconscious by some indicators. The outcome of consciousness of the indicators could not be assessed on conscious animals in these studies, as all animals were already unconscious at the time of testing post stun. The systematic review revealed that many studies (so-called ‘prevalence studies’) report the proportion of stunned animals with outcomes of consciousness or unconsciousness, rather than the proportion of truly unconscious or conscious animals at a set time point with the outcome of the indicators. Such data cannot be translated into sensitivity and specificity. However, prevalence studies were used to describe the indicators in section 3.2 of this opinion.

Table 6 summarises the information on the characteristics of the indicators derived from the systematic review.

[12] Indicators used to check the state of consciousness and unconsciousness.
[13] Indicators used to check the state of life and death.

Table 6: Information on the characteristics of the indicators derived from the systematic review.

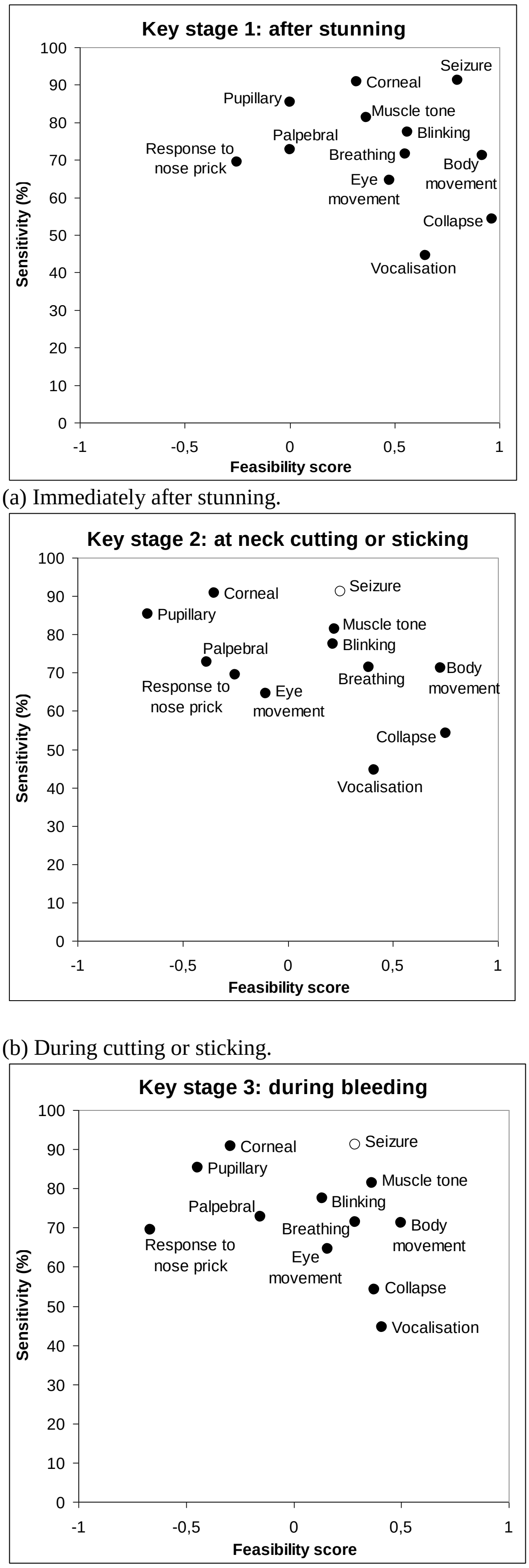
References	Species	Stun method	Outcome of unconsciousness of the indicator	Number of animals defined as unconscious	Number of animals tested unconscious by the indicator	Percentage unconscious animals tested unconscious
Blackmore and Newhook, 1982	Bovine	Captive bolt	No palpebral reflex	2	2	100
Blackmore and Newhook, 1982	Bovine	Captive bolt	No corneal reflex	2	2	100
Blackmore and Newhook, 1982	Bovine	Captive bolt	No tonic seizure	2	2	100
Lambooij and Spanjaard, 1981	Bovine	Captive bolt	No corneal reflex	23	23	100
Lambooij and Spanjaard, 1981	Bovine	Captive bolt	No tonic seizure	23	23	100

3.3. Results from questionnaire 2

From the second questionnaire, namely the online survey, answers from around 82 experts were collected. Respondents could answer for more than one species or method, depending upon their work experience, so the total number of completed surveys was 84.

In total, 36 respondents said that they monitor the welfare of bovines following captive bolt stunning. Out of these 36 respondents, 34 answered the feasibility as well as the sensitivity/specificity question. Of these, 32 experts provided 342 answers eligible for analysis (see section 2.2.3).

Figure 3a, b and c combine the feasibility score and estimate of sensitivity for each indicator for captive bolt stunning at each key stage (key stage 1 = immediately after stunning, key stage 2 = at neck cutting or sticking, key stage 3 = during bleeding). Thus, indicators nearest the top-right corner have high sensitivity and high feasibility. In all three graphs the sensitivity value is identical but the feasibility score changes according to the respondent ratings. Empty symbols in (b) and (c) mark indicators that are considered as not applicable during that stage although the respondents did evaluate their feasibility.



(a) Immediately after stunning.

(b) During cutting or sticking.

(c) During bleeding.

Figure 3: Graphical combination of feasibility score and sensitivity resulting from questionnaire 2 for each indicator at (a) key stage 1 = immediately after stunning, (b) key stage 2 = during cutting or sticking and (c) key stage 3 = during bleeding

Twelve respondents said that they are experienced with slaughter without stunning in bovines. The respondents reported the feasibility of assessing indicators prior to release from restraint and carcase dressing. Specificity of most indicators, i.e. the ability to confirm an animal becoming unconscious or dead, was estimated greater than 90% throughout all indicators and was greater than 95% in 12 out of the 13 estimates (see Table 8 and 9).

Figure 4 combines the feasibility score and estimates of sensitivity for indicators applied prior to release from restraint and indicators applied prior to carcase dressing.

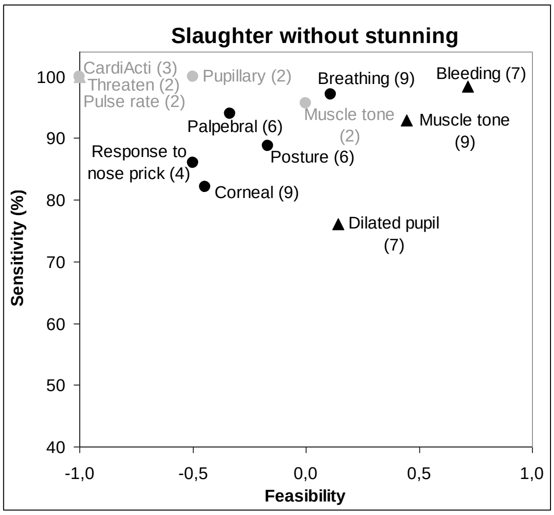


Figure 4: Graphical combination of feasibility score and sensitivity resulting from questionnaire 2 for each indicator prior to release from restraint (circles) and prior to dressing (triangles). Grey symbols/items are indicators with a minimum number of data points

The prominent indicators were (circles—prior to release) breathing, muscle tone and posture and (triangles—prior to dressing) bleeding, relaxed body and dilated pupils. The indicators applied prior to release from restraint are generally less than ideal as there was no indicator that combined high feasibility score with high sensitivity value.

3.4. Description of indicators for captive bolt stunning and overview of their performance

The combined efforts of the above activities led to the following overview of indicators and outcomes of consciousness and unconsciousness.

The following paragraphs discuss the indicators and their outcomes mentioned above in relation to their relevance in identifying consciousness at key stages of monitoring slaughter with penetrative captive bolt stunning. Some of these outcomes occur spontaneously following stunning (e.g. collapse of the animal without regaining posture) and/or sticking, whereas some other outcomes will have to be intentionally provoked (e.g. corneal reflex). The Working Group agreed that the risk of poor welfare can be detected better if animal welfare monitoring is focused on detecting consciousness. The presence of certain outcomes (e.g. vocalisation) or a positive response of the animal to an applied stimulus (e.g. corneal reflex) is most relevant. In addition, the sensitivity, specificity and feasibility of the indicators are presented, based on information gathered in the different activities described in this opinion. Depending on all these aspects, some indicators may not be applicable to monitoring at certain key stages.

3.4.1. Posture

3.4.1.1. Description

Effective stunning will result in brain concussion, which can be recognised from the immediate and permanent loss of posture described in this opinion as **immediate collapse** of the animal to the floor; therefore, in key stage 1 (i.e. after stunning), unconsciousness manifests as immediate collapse of the animal and, if the captive bolt stunning is ineffective, the animal will either **fail to collapse** or **attempt to regain posture** within the stunning box. If stunning is ineffective (e.g. because shooting position is incorrect), the animals may lose posture as a result of the impact of the bolt and head injury and remain collapsed in the restraining/stunning box, without making any attempt to regain posture, which may be detected at the time of rolling out of the stunning box. Bovines showing signs of ineffective captive bolt stunning will require immediate re-stun.

A bovine that has been effectively stunned with a captive bolt gun will be shackled, hoisted and presented for sticking or neck cutting, which is key stage 2. An unconscious bovine at this stage will be hanging flaccidly on the overhead shackle and is therefore not expected to show any **changes in its posture.** A bovine recovering consciousness whilst hanging on the overhead shackle (during key stages 2 and 3) will **attempt to regain posture**, which will be manifested as **arching of the neck or body**; such an animal will have to be re-stunned.

3.4.1.2. Feasibility

In questionnaire 2 immediate collapse was rated as an outcome of unconsciousness that is easy or normal to assess at stunning (key stage 1) by 96 % and 4 % respectively.

3.4.1.3. Sensitivity and specificity

The outcome of consciousness of ‘posture’ is the maintenance of an upright posture. Therefore, the sensitivity is the percentage of animals which maintain upright posture immediately after stunning, out of all truly conscious animals. This was estimated by questionnaire 2 respondents to be 54 % ($n = 25$; for data range, see Table 7). The specificity is calculated as the percentage of animals immediately loosing posture, out of all truly unconscious animals. This was estimated to be 98 % ($n = 26$). The reason for the low sensitivity is that immediate loss of posture without loss of consciousness might be present as a result of the impact of the bolt on the head. In these cases, animals may or may not try to regain posture.

3.4.2. Breathing

3.4.2.1. Description

In key stage 1, effective captive bolt stunning of bovine will lead to **immediate onset of apnoea** (absence of breathing), which can be used to monitor the effectiveness of stunning. Ineffective captive bolt stunning can be recognised from the sustained **presence of breathing**, including **laboured breathing**. Animals that recover consciousness will start to breathe normally, in a pattern commonly referred to as **rhythmic breathing**, which involves a respiratory cycle of inspiration and expiration. Rhythmic breathing can be recognised from the regular flank and/or mouth and nostril movements. **Recovery of breathing**, if not apparent from these movements, can be determined by holding a small mirror in front of the nostrils or mouth, as breathing will cause condensation to appear on the mirror as a result of the expiration of moist air. A bovine recovering consciousness whilst hanging on the overhead shackle will attempt to breathe, which may begin as **regular gagging** and progress to **resumption of breathing**; such an animal will have to be re-stunned. An effectively stunned and stuck bovine will remain unconscious until death occurs in key stage 3 and therefore is not expected to show any signs of breathing.

3.4.2.2. Feasibility

Breathing was rated as easy and normal to assess at stunning by 62 % and 31 %, respectively; at sticking by 50 % and 38 %, respectively; and during bleeding by46 % and 36 %, respectively. This is probably because it may not be possible to recognise or assess breathing in animals shackled and hoisted on to the overhead rail. Two experts considered breathing as not applicable at neck cutting or sticking.

3.4.2.3. Sensitivity and specificity

The positive outcome of breathing is the sign of consciousness, namely the resumption of breathing. Therefore, the sensitivity is the percentage of animals which show presence of breathing, out of all truly conscious animals. This was estimated by questionnaire 2 respondents to be 72 % (n = 24). The specificity is calculated as the percentage of animals showing apnoea, out of all truly unconscious animals. This was estimated to be 97 % (n = 27). The reason for the low sensitivity was not described. However, responders might find it difficult to differentiate when gagging becomes regular breathing and leads to recovery of consciousness.

3.4.3. Tonic seizure

3.4.3.1. Description

In key stage 1, effective captive bolt stunning leads to **onset of tonic seizure** soon after collapse, which may be recognised from the occurrence of an arched back and rigidly flexed legs under the body. It may be followed by clonic seizure manifesting as leg kicking or paddling. Tonic seizure may not always be recognised, especially when a stunning box or restraint is used.

The tonic seizure lasts for several seconds but leads to loss of muscle tone, usually before the animal is rolled out of the stun box, shackled, hoisted and presented for sticking. It may end, and therefore not be detected, if there is a long delay between the captive bolt stunning and the removal of the animal from the restraining/stunning box. Therefore, tonic seizure as an indicator is not applicable at key stages 2 and 3.

3.4.3.2. Feasibility

From questionnaire 2, tonic seizure was rated as easy to assess at stunning by 80 % of experts. One expert considered tonic seizure as not applicable at neck cutting and two as not applicable during bleeding.

3.4.3.3. Sensitivity and specificity

- From the systematic review (SR) report: Two studies provided data suggesting that the presence of seizure (one study did not specify the type of seizure) was a 100 % specific outcome of unconsciousness in the study animals, i.e. unconscious animals have seizures in the setting of stun-kill studies. One study (Lambooij et al., 1981) reported narratively that unconsciousness and seizures occurred immediately in all animals. The other study (Blackmore and Newhook, 1982) reported that the onset of unconsciousness occurred 12–15 seconds post stun in the two animals studied; both animals had seizures. The systematic review concluded that the authors did not provide enough data to estimate the sensitivity of tonic seizure. The Working Group's interpretation was that presence of seizure is a clear outcome of unconsciousness due to brain concussion, and further evidence indicated that, even in the absence of visible changes in the spontaneous EEG, visually evoked potentials (VEPs) in the brain were abolished immediately after captive bolt stunning in cattle (Daly et al., 1987, 1988).

- From questionnaire 2: The positive outcome of tonic seizure is the sign of consciousness, namely the absence of tonic seizures. Therefore, the sensitivity is the percentage of animals which do not show the onset of tonic seizures immediately after stunning, out of all truly conscious animals. This was estimated by questionnaire 2 respondents to be 91 % (*n* = 9). The specificity is calculated as the percentage of animals showing onset of tonic seizures, out of all truly unconscious animals. This was estimated to be 97 % (*n* = 10).

3.4.4. Muscle tone

3.4.4.1. Description

Effectively stunned animals will show tonic/clonic seizure followed by general **loss of muscle tone**, which can be recognised from **completely relaxed legs**, **floppy ears and tail**, **relaxed jaw** and **protrusion of tongue**. These signs are especially visible when the animals are hanging from the overhead rail at key stages 2 and 3, and therefore muscle tone is not applicable at key stage 1. On the other hand, conscious animals at key stages 2 and 3 retain or recover certain levels of **muscle tone** manifested as **stiff ears and jaws**, **arched tail**, **retraction of tongue into the mouth** and **flexing of forelegs**. Animals showing any of these signs of muscle tone must be re-stunned. Ineffectively stunned bovines and those recovering consciousness will attempt to raise their heads during the three key stages of monitoring, which is referred to as **righting reflex** in this opinion, and bovines showing this reflex will have to be re-stunned.

3.4.4.2. Feasibility

From questionnaire 2, muscle tone was rated as easy to assess at stunning by 45 %, at sticking by 33 % and during bleeding by 45 % of experts. Muscle tone was considered as normal to assess by 45 % at stunning, by 56 % at sticking and by 45 % during bleeding. About 10 % of experts considered muscle tone as difficult, i.e. after stunning (9 %), at neck cutting or sticking (11 %) and during bleeding (9 %). Two experts considered muscle tone as not applicable at neck cutting/sticking.

3.4.4.3. Sensitivity and specificity

The positive outcome of muscle tone, namely the presence of muscle tone, is the sign of consciousness. Therefore, the sensitivity is the percentage of animals which show a certain level of muscle tone, out of all truly conscious animals. This was estimated by questionnaire 2 respondents to be 81 % (*n* = 9). The specificity is calculated as the percentage of animals observed to show loss of muscle tone, out of all truly unconscious animals. This was estimated to be 75 % (*n* = 11). The percentage of conscious animals estimated not to show muscle tone was 19 %; this could reflect the fact that the respondents did not recognise muscle tone as such or because the interpretation of muscle tone may be different across respondents.

3.4.5. Response to nose prick or ear pinch

3.4.5.1. Description

Ineffective captive bolt stunning and recovery of consciousness as a result of poor stunning can be recognised from the **response to nose prick or ear pinch** with a sharp instrument at all key stages of monitoring. Animals showing a positive response to a painful stimulus at any stage must be re-stunned. In contrast, effective captive bolt stunning of bovine may lead to damage to the region of the brain (somatosensory cortex) associated with the perception of nociception, and therefore the animal is not expected to show response to a painful stimulus such as a pin prick to the muzzle (area between external nostrils) or the ear at key stages 1, 2 and 3.

3.4.5.2. Feasibility

From questionnaire 2, response to nose prick or ear pinch (only four respondents) was considered as easy or normal to assess by, respectively, 25 % and 25 % at stunning, 0 % and 75 % at sticking and 0 % and 33 % during bleeding. Some experts reported that response to nose prick or ear pinch is difficult at any of the key stages of monitoring. The reason why a response to a painful stimulus, i.e. nose prick or ear pinch, could not be elicited was not described in the survey results.

3.4.5.3. Sensitivity and specificity

The positive outcome of the response to nose prick or ear pinch is the sign of consciousness, namely a positive response to nose prick or ear pinch. Therefore, the sensitivity is the percentage of animals which do respond to nose prick or ear pinch, out of all truly conscious animals. This was estimated by questionnaire 2 respondents to be 70 % ($n = 4$). The specificity is calculated as the percentage of animals showing no response to nose prick or ear pinch, out of all truly unconscious animals. This was estimated to be 91 % ($n = 4$). The reduced sensitivity is because the lack of response to painful stimulus is associated with the inability of the animal to perceive them.

3.4.6. Body movements in response to sticking

3.4.6.1. Description

Body movements in response to sticking include intentional or purposeful **kicking** and **body or head movements** that may occur as a nociceptive response to incision of the skin for the purpose of bleeding and/or insertion of the knife for the purpose of sticking or neck cutting. Therefore, the indicator body movements for checking for the state of consciousness is applicable only to key stage 2. The difficulty with the indicator is that some unconscious animals can react to sticking, and this mainly takes the form of movement of the forelegs, which is referred to in the scientific literature as the somatic reflex arc (other examples include the patellar reflex or knee jerk response) and such reflexes do not involve the central nervous system. Nevertheless, when such a reflex is seen during sticking, ‘personnel’ should check for other signs to rule out persistence of consciousness.

3.4.6.2. Feasibility

From questionnaire 2, body movements, defined in this opinion as kicking after stunning and at sticking, were considered by all experts as easy or normal to assess. One expert considered body movements as not applicable at sticking.

3.4.6.3. Sensitivity and specificity

The positive outcome of body movement is the sign of consciousness, namely occurrence of kicking and body or head movements. Therefore, the sensitivity is the percentage of animals which move immediately after stunning, out of all truly conscious animals. This was estimated by questionnaire 2 respondents to be 71 % ($n = 10$). The specificity is calculated as the percentage of animals showing no body movement, out of all truly unconscious animals. This was estimated to be 28 % ($n = 10$). The extreme low estimate of specificity may reflect the fact that some kind of body movement is expected in most unconscious animals and thus the questionnaire may have insufficiently defined the positive outcome of the indicator body movements.

3.4.7. Vocalisation

3.4.7.1. Description

Conscious animals may vocalise, and therefore purposeful **vocalisation**, such as grunting, bellowing or mooing, can be used to recognise ineffective stunning in key stage 1, and recovery of consciousness in key stages 2 and 3. However, not all conscious animals will vocalise. Nevertheless, vocalisation is routinely used in slaughterhouses to detect consciousness following captive bolt stunning in bovines. Animals showing vocalisation must be re-stunned.

3.4.7.2. Feasibility

From questionnaire 2, vocalisation was considered to be easy or normal to assess by 71 % and 24 %, respectively, at stunning, by 65 % and 12 %, respectively, at sticking and by 53 % and 35 %, respectively, during bleeding. Some experts considered checking vocalisation as difficult at any of the key stages (1, 5 %; 2, 23 %; 3, 12 %), mostly at sticking.

3.4.7.3. Sensitivity and specificity

The positive outcome of vocalisation is the sign of consciousness, namely the presence of vocalisation. Therefore, the sensitivity is the percentage of animals which vocalise after stunning, out of all truly conscious animals. This was estimated by questionnaire 2 respondents to be 45 % ($n = 14$). The specificity is calculated as the percentage of animals showing no vocalisation, out of all truly unconscious animals. This was estimated to be 98 % ($n = 16$). The reason for the extremely low sensitivity is, as mentioned before, that not all conscious animals necessarily vocalise.

3.4.8. Eye movements

3.4.8.1. Description

Eye movements and the position of the eyeball can be recognised from close examination of eyes after captive bolt stunning. In key stage 1, effective captive bolt stunning of bovines will produce **fixed eyes** as a result of concussion of the brain, and this can be recognised by eyes that are wide open and glassy, with clearly visible iris/cornea in the middle, and which remain so until death occurs. Bovines that are not effectively stunned with captive bolt or those recovering consciousness will show **eye movements, including nystagmus** (spontaneous rapid, side-to-side movements of the eyeballs) or rotation of the eyeball. Rotation of the eyeball can be recognised from the appearance of mostly sclera, with little or no iris/cornea being visible. Eye movements can be used to recognise consciousness during all the three key stages; and animals showing any eye movements must be re-stunned.

3.4.8.2. Feasibility

From questionnaire 2, eye movements were considered as easy to assess at stunning by 62 % of experts, at sticking by 37 % and during bleeding by 42 %. Eye movements were rated as normal to assess at stunning by 24 %, at sticking by 16 % and during bleeding by 32 % of experts. Checking for eye movements was rated as difficult at stunning by 14 %, at sticking by 47 % and during bleeding by 26 % of experts. It may be difficult or impossible to observe eye movements at the time of sticking or bleeding for personnel facing the brisket. One expert considered eye movements as not applicable at sticking and during bleeding.

3.4.8.3. Sensitivity and specificity

The positive outcome of eye movements is the sign of consciousness, namely the presence of eye movements. Therefore, the sensitivity is the percentage of animals which show eye movements, out of all truly conscious animals after stunning. This was estimated by questionnaire 2 respondents to be 65 % ($n = 19$). The specificity is calculated as the percentage of animals observed to show fixed eyes, out of all truly unconscious animals. This was estimated to be 95 % ($n = 20$). The correlation between the expert ratings of feasibility and sensitivity was about only20 % ; hence the low sensitivity is rather associated with the limited number of conscious animals that after stunning may show eye movements.

3.4.9. Palpebral reflex

3.4.9.1. Description

The palpebral reflex is a blinking response elicited by touching or tapping a finger on the inner/outer eye canthus or eyelashes. Effective captive bolt stunning leads to abolition of the palpebral reflex. Effectively stunned and stuck animals show **no palpebral reflex** during any key stage. In contrast, ineffectively stunned animals and those recovering consciousness prior to sticking or during bleeding are expected to show a palpebral reflex at any key stage. Animals showing a positive palpebral reflex must be re-stunned.

3.4.9.2. Feasibility

From questionnaire 2, the palpebral reflex was rated as easy and normal to assess at stunning by 29 % and 43 %, respectively, at sticking by 15 % and 31 %, respectively, and during bleeding by 38 % and 8 %, respectively, of experts. A number of experts considered the palpebral reflex as difficult to assess at stunning (29 %), at sticking (54 %) and during bleeding (54 %), and one expert considered palpebral reflexes as not applicable at sticking and during bleeding. It is possible that inaccessibility/lack of access to animals during bleeding was taken into consideration for rating eye reflexes as difficult (palpebral and corneal reflexes and spontaneous blinking).

3.4.9.3. Sensitivity and specificity

- From the systematic review report: Blackmore and Newhook (1982) reported that the palpebral reflex was absent in both calves in the first 20 seconds post stun and that the same animals were unconscious. Therefore, this indicator is 100 % specific. The systematic review concluded that the authors did not provide enough data to estimate the sensitivity of the palpebral reflex.

- From questionnaire 2: The positive outcome of palpebral reflex is the sign of consciousness, namely the present palpebral reflex. Therefore, the sensitivity is the percentage of animals which show a palpebral reflex, out of all truly conscious animals after stunning. This was estimated by questionnaire 2 respondents to be 73 % (n = 12). The specificity is calculated as the percentage of animals showing no palpebral reflex, out of all truly unconscious animals. This was estimated to be 96 % (n = 13).

3.4.10. Corneal reflex

3.4.10.1. Description

The corneal reflex is a blinking response elicited by touching or tapping the cornea with a finger or paint brush. Effective captive bolt stunning leads to permanent abolition of corneal reflex. Effectively stunned and stuck animals show **no corneal reflex** during any key stage. In contrast, ineffectively stunned animals and those recovering consciousness prior to sticking or during bleeding are expected to show a corneal reflex at any key stage. Animals showing a corneal reflex must be re-stunned.

3.4.10.2. Feasibility

From questionnaire 2, the corneal reflex was rated as easy to assess at stunning by 52 %, at sticking by 15 % and during bleeding by 21 % of experts. The corneal reflex was rated as normal to assess at stunning by 28 %, at sticking by 35 % and during bleeding by 29 % of experts. A number of experts also considered the corneal reflex as difficult to assess at stunning (20 %), at sticking (50 %) and during bleeding (50 %; a further three said it is not applicable).

It is possible that inaccessibility/lack of access to animals during bleeding was taken into consideration for rating eye reflexes as difficult to assess (palpebral and corneal reflexes and spontaneous blinking).

3.4.10.3. Sensitivity and specificity

- From the systematic review report: Two studies assessed the absence of corneal reflex in unconscious animals. These data suggest that the corneal reflex is absent in unconscious calves stunned using a captive bolt. Lambooij et al. (1981) reported that immediate abolition of the corneal reflex and ‘immediate’ onset of unconsciousness occurred in the 23 veal calves. Blackmore and Newhook (1982) reported that EEG changes indicative of unconsciousness occurred within 12–15 seconds in two calves but the corneal reflex could not be evoked in the first 20 seconds after stun. This would suggest 100 % specificity for the corneal reflex, i.e. unconscious animals stunned using captive bolt do not exhibit a corneal reflex. With respect to sensitivity, the results from the two conscious animals are unclear, but if taken exactly as reported would suggest low sensitivity, i.e. conscious animals also had no corneal reflex because the animals were reported as conscious based on EEG for 12–15 seconds but did not have a corneal reflex at any time in the first 20 seconds. Realistically it is hard to know how often, if at all, the corneal reflex could have been tested in the first 10 seconds. The systematic review concluded that the authors did not provide enough data to estimate the sensitivity of the corneal reflex.

- From questionnaire 2: The positive outcome of the corneal reflex is the sign of consciousness, namely the presence of a corneal reflex. Therefore, the sensitivity is the percentage of animals showing a corneal reflex, out of all conscious animals after stunning. This was estimated by questionnaire 2 respondents to be 91 % (*n* = 19). The specificity is calculated as the percentage of animals observed to show no corneal reflex, out of all truly unconscious animals. This was estimated to be 97 % (*n* = 23). The reason for the high sensitivity is that captive bolt stunning which failed to induce unconsciousness may have improperly damaged the part of the brain stem controlling the corneal reflex, resulting in the presence of a corneal reflex in an animal conscious after stunning.

3.4.11. Blinking

3.4.11.1. Description

Spontaneous blinking, opening and closing of the eyelids (fast or slowly) without stimulation, is expected only in conscious animals and can be used as an outcome of consciousness in all key stages of monitoring. However, not all conscious animals will show spontaneous blinking, and hence absence of blinking does not always mean that the animal is unconscious. Animals showing blinking must be re-stunned.

3.4.11.2. Feasibility

From questionnaire 2, spontaneous blinking was rated as easy to assess at stunning by 63 %, at sticking by 50 % and during bleeding by 33 % of experts. Spontaneous blinking was rated as normal at stunning by 31 %, at sticking by 21 % and during bleeding by 47 % of experts. Some experts also considered spontaneous blinking as difficult at stunning (6 %), at sticking (29 %) and during bleeding (20 %), and one expert said spontaneous blinking is not applicable in the later stages. It is possible to suggest that inaccessibility/lack of access to animals during bleeding has been taken into consideration for rating eye reflexes as difficult (palpebral and corneal reflexes and spontaneous blinking).

3.4.11.3. Sensitivity and specificity

The positive outcome of blinking is the sign of consciousness, namely the presence of spontaneous blinking. Therefore, the sensitivity is the percentage of animals which show spontaneous blinking, out of all conscious animals after stunning. This was estimated by questionnaire 2 respondents to be 77 % (*n* = 13). The specificity is calculated as the percentage of animals observed to show no spontaneous blinking, out of all unconscious animals. This was estimated to be 98 % (*n* = 14). The reason for the medium sensitivity may be that spontaneous blinking is not instigated during the check and thus may be absent also in conscious animals.

3.4.12. Pupillary reflex

3.4.12.1. Description

The pupillary reflex is constriction of the pupils (miosis) in response to focusing or shining a torch at the pupils. Effective captive bolt stunning leads to abolition of the pupillary reflex. Effectively stunned and stuck animals show **no pupillary reflex** during any key stage. In contrast, ineffectively stunned animals and those recovering consciousness prior to sticking or during bleeding are expected to show a pupillary reflex at any key stage. Animals showing the pupillary reflex must be re-stunned.

3.4.12.2. Feasibility

From questionnaire 2, the pupillary reflex was rated as easy to assess at stunning by 36 %, at sticking by 0 % and during bleeding by 11 % of experts. The pupillary reflex was rated as normal to assess at stunning by 27 %, at sticking by 33 % and during bleeding by 33 % of experts. The majority of experts considered the pupillary reflex as difficult to assess at stunning (36 %), at sticking (67 %) and during bleeding (56 %), and one expert said pupillary reflex is not applicable in the later stages. The main reason for these ratings could be the lack of access to animals.

3.4.12.3. Sensitivity and specificity

The positive outcome of pupillary reflex is the sign of consciousness, namely the presence of pupillary reflex. Therefore, the sensitivity is the percentage of animals which show pupillary reflex out of all truly conscious animals after stunning. This was estimated by questionnaire 2 respondents to be 85 % (n = 8). The specificity is calculated as the percentage of animals showing no pupillary reflex, out of all truly unconscious animals. This was estimated to be 97 % (n = 9). The potential reason for the high sensitivity is that captive bolt stunning which failed to induce unconsciousness may have improperly damaged the part of the brain stem controlling the pupillary reflex, resulting in the presence of pupillary reflex in an animal conscious after stunning.

A summary of the information on indicator sensitivity, specificity and feasibility collected from questionnaire 2 and the systematic literature review is presented in Table 7.

Table 7: Summary of information on the sensitivity, specificity and feasibility of indicators and outcomes of consciousness from questionnaire 2 and systematic review

Indicators after captive bolt stunning	**Outcomes of consciousness**	**Sensitivity (%)**	**Data (without uncertainty), average (20th, 50th and 80th percentiles)**	**Specificity (%)**	**Data (without uncertainty), average (20th, 50th and 80th percentiles)**	**Feasibility**		
						After stunning	**During neck cutting**	**During bleeding**
Posture [(a)]	No collapse or attempts to regain posture	54	53 (10, 50, 90)	98	98 (95, 100, 100)	0.96	0.75	0.38
Breathing	Presence of breathing	72	71 (43; 93; 100)	97	97 (95, 100, 100)	0.55	0.38	0.29
Tonic seizure [(b)]	Absence of tonic seizures	91	9 (95, 100, 100)	100 (SR 97)	97 (95, 100, 100	0.80	0.25	0.29
Muscle tone	Presence of muscle tone	81	84 (80, 100, 100)	75	70 (10, 95, 100)	0.36	0.22	0.36
Response to nose prick or ear pinch	Positive response	70	70 (n.a.)	92	91 (n.a.)	–0.25	–0.25	–0.67
Body movements	Presence of movements	71	72 (55, 90, 100)	28	33 (0, 0, 80	0.92	0.73	0.50
Vocalisation	Presence of vocalisation	45	46 (21, 30, 88)	98	98 (95, 99, 100)	0.65	0.41	0.41
Eye movements	Presence of eye movements	65	65 (45, 85, 100)	95	94 (89, 95, 100)	0.48	–0.11	0.16
Palpebral reflex	Presence of reflex	73	79 (75, 100, 100)	100 (SR 96)	96 (92, 98, 100)	0.00	–0.38	–0.15
Corneal reflex	Presence of reflex	91	91 (98, 100, 100)	100 (SR 97)	97 (94, 98, 100)	0.32	–0.35	–0.29
Spontaneous blinking	Presence of blinking	77	79 (60, 90, 100)	98	97 (94, 99, 100)	0.56	0.21	0.13
Pupillary reflex	Presence of reflex	85	84 (80, 100, 100)	97	96 (93, 95, 100)	0.00	–0.67	–0.44

(a): In questionnaire 2, posture as an indicator was referred to its outcome of unconsciousness, namely ‘immediate collapse’.

(b): In questionnaire 2, the question about ‘tonic seizures’ addressed the outcome of unconsciousness, namely the ‘presence of tonic seizures’.

n.a., not applicable as only four respondents provided data (10, 70, 98, 100; and 75, 95, 96 and 99). SR, systematic review.

3.5. Description of indicators for slaughter without stunning and overview of their performance

Two separate sets of indicators and outcomes are proposed, one for determination of unconsciousness and one for the determination of death. This is mainly because indicators used to determine unconsciousness may not be relevant to death. In addition, Regulation 1099/2009 requires that unconsciousness be established prior to releasing animals from the restraint, while death must be established in animals prior to dressing.

In the first phase of slaughter without stunning, the focus is on the determination of the onset of unconsciousness through the outcome of the tested indicators (specificity) while the monitoring focus is on animals still conscious (sensitivity). In the second phase of slaughter without stunning, the focus is on the determination of onset of death through the outcome of tested indicators suggestive of death (specificity) while the monitoring focus is on animals still live (sensitivity).

The systematic review concluded that no studies reported the use of an EEG-based measure of death. Three studies reported the use of EEG as a means of establishing unconsciousness or insensibility as gold standard for other outcomes of unconsciousness (Newhook and Blackmore, 1982; Cook et al., 1996; Lambooij et al., 2012) but these findings were not relevant for the review; thus, no further discussion is presented in this scientific opinion.

3.5.1. Indicators prior to release from restraint

This list of indicators to be used to confirm unconsciousness is intended for use before the animal is released from the restraints. For slaughter without stunning, the specific estimates of feasibility and sensitivity/specificity from questionnaire 2 have to be considered with caution because only 12 respondents addressed this method of slaughter, of whom only 10 answered the feasibility and sensitivity/specificity questions.

3.5.1.1. Posture

Description

Permanent loss of posture can be used as the earliest physical sign of the onset of unconsciousness following neck cutting of an unrestrained/free-standing animal. It is inevitable that slaughter without stunning will lead to **loss of posture** provided the neck cutting was swift and bleeding was profuse and uninterrupted. **Loss of posture** (or **loss of balance**) can be used only in bovines which are free standing or lightly restrained in the upright position. Therefore, loss of posture cannot be determined in animals that are severely restrained and/or rotated. Caution is required as animals may fall down after slaughter without stunning if the floor is slippery, or they may continue to struggle to stand up, again without success, or simply lie down. For this reason, close and continuous observation of bovines is required to ensure that the loss of posture is a true occurrence. Some animals may exhibit loss of posture as a result of the loss of significant proportions of circulating blood volume but subsequently suffer carotid artery occlusion and recover consciousness (Gregory et al., 2010). However, these animals may or may not attempt to regain posture. Therefore, complete and **permanent loss of posture without attempt to regain posture** can be used as an outcome of unconsciousness.

Feasibility

From questionnaire 2, loss of posture after neck cutting as an outcome of an indicator was rated (n = 6) as easy to assess by 33 % and as normal to assess by 17 %. However, 50 % of experts considered loss of posture difficult to assess. This is probably because loss of posture can be assessed only when the animal is free standing or lightly restrained in the upright position. Loss of posture cannot be assessed in cattle that are severely restrained and/or rotated, as they do not have freedom of movement to collapse.

Sensitivity and specificity

The outcome of unconsciousness of the indicator ‘posture’ is the loss of posture or collapse. Therefore, the specificity is the percentage of unconscious animals which lose posture after neck cutting, out of all truly unconscious animals. This was estimated by questionnaire 2 respondents to be 99 % (*n* = 6). The sensitivity is calculated as the percentage of conscious animals that do not show collapse, out of all truly conscious animals. This was estimated to be 89 % (*n* = 6). The reason for the reduced sensitivity is that conscious animals might collapse for reasons other than loss of consciousness (e.g. slippery floor owing to the presence of blood). Nevertheless, the specificity is very high because an unconscious animal has to lose its posture.

3.5.1.2. Muscle tone

Description

Loss of a significant proportion of circulating blood volume is also expected to result in general loss of muscle tone, resulting in relaxed body, which can be used to recognise the onset of unconsciousness. Involuntary muscle jerks may occur occasionally. Depending upon the type and severity of restraint, **loss of tone in neck and leg muscles** could be used as an outcome of unconsciousness. Loss of muscle tone is related to the loss of posture, and therefore might be difficult to assess when the animals are held in a restraint or rotated.

Feasibility

From questionnaire 2, muscle tone was rated (*n* = 2) as normal to assess by 100 %.

Sensitivity and specificity

The outcome of unconsciousness of the indicator ‘muscle tone’ is the loss of muscle tone. Therefore, the specificity is the percentage of unconscious animals without muscle tone after neck cutting, out of all truly unconscious animals. This was estimated by questionnaire 2 respondents to be 100 % (*n* = 3). The sensitivity is calculated as the percentage of conscious animals showing a certain level of muscle tone, out of all conscious animals. This was estimated to be 96 % (*n* = 3).

3.5.1.3. Breathing

Description

Loss of consciousness following neck cutting will eventually lead to cessation of rhythmic breathing. **Rhythmic breathing** can be recognised from the regular flank movement. Therefore, sustained absence of rhythmic breathing (absence of a respiratory cycle—inspiratory and expiratory movements) can be used as an outcome of unconsciousness. **Cessation of breathing** can also be used as an outcome of unconsciousness in animals. However, since the trachea is also severed at the time of neck cutting at slaughter without stunning, absence of breathing cannot be assessed from the air movement at the external nostrils and will have to be confirmed by the absence of air bubbles in the liquid blood or sera accumulating at the neck wound or by the absence of any flank movements suggestive of breathing.

Feasibility

From questionnaire 2, breathing was rated (*n* = 9) as easy to assess by 33 % and as normal to assess by 44 %. However, 22 % of experts considered breathing as difficult to assess prior to release from restraint. This is probably because breathing is difficult to recognise through the flank movements when the animal is severely restrained or rotated and cannot be ascertained through the nose if the trachea has been severed.

Sensitivity and specificity

The outcome of unconsciousness of the indicator ‘breathing’ is the immediate onset of apnoea. Therefore, the specificity is the percentage of unconscious animals which show apnoea after neck cutting, out of all animals truly unconscious. This was estimated by questionnaire 2 respondents to be 92 % (n = 9). The sensitivity is calculated as the percentage of conscious animals showing breathing, out of all conscious animals. This was estimated to be 97 % (n = 9).

3.5.1.4. Pupillary reflex

Description

The pupillary reflex is constriction of the pupils (miosis) in response to focusing or shining a torch at the pupils and will inevitably be abolished in unconscious animals. Therefore, unconsciousness following neck cutting during slaughter without stunning can be determined from the **absence of the pupillary reflex**. On the other hand, animals recovering consciousness are expected to show a pupillary reflex.

Feasibility

From questionnaire 2, the pupillary reflex was rated (n = 2) as either normal or difficult to assess (one respondent each).

The reason for these ratings is probably the lack of access to the animal and the fact that eyes are usually covered with blood, especially when the animals are rotated on to their backs.

Sensitivity and specificity

The outcome of unconsciousness of the indicator ‘pupillary reflex’ is absence of the pupillary reflex. Therefore, the specificity is the percentage of animals which do not show pupillary reflex after neck cutting, out of all animals truly unconscious. This was estimated by questionnaire 2 respondents to be 98 % (n = 2). The sensitivity is calculated as the percentage of conscious animals showing a positive pupillary reflex, out of all truly conscious animals. This was estimated to be 100 % (n = 2).

3.5.1.5. Palpebral reflex

Description

The palpebral reflex is a blinking response elicited by touching or tapping a finger on the inner/outer eye canthus or eyelashes and will inevitably be abolished in unconscious animals. Therefore, unconsciousness following neck cutting during slaughter without stunning can be determined from the **absence of the palpebral reflex**. In contrast, animals recovering consciousness during bleeding are expected to show a palpebral reflex.

Feasibility

From questionnaire 2, the palpebral reflex was rated (n = 6) as easy to assess by 17 %, as normal to assess by 33 % and as difficult to assess by 50 %. The reason for these ratings is probably the lack of access to the animal and the fact that eyes are usually covered with blood, especially when the animals are rotated on to their backs.

Sensitivity and specificity

The outcome of unconsciousness of the indicator ‘palpebral reflex’ is the absence of a palpebral reflex. Therefore, the specificity is the percentage of unconscious animals showing no palpebral reflex after neck cutting, out of all animals truly unconscious. This was estimated by questionnaire 2 respondents to be 100 % (n = 3). The sensitivity is calculated as the percentage of conscious animals blinking in response to stimulus of the palpebrae, out of all truly conscious animals. This was estimated to be 94 % (n = 5).

3.5.1.6. Corneal reflex

Description

The corneal reflex is a blinking response elicited by touching or tapping the cornea, and will inevitably be abolished in unconscious animals. Therefore, unconsciousness following neck cutting during slaughter without stunning can be determined from the **absence of the corneal reflex**. In contrast, animals recovering consciousness during bleeding are expected to show a corneal reflex.

Feasibility

From questionnaire 2, the corneal reflex was rated (*n* = 9) as easy to assess by 11 % and as normal to asses by 33 %. Half of the respondents (56 %) considered the corneal reflex difficult to assess.

The reason for these ratings is probably the lack of access to the animal and the fact that the eyes are usually covered with blood, especially when the animals are rotated on to their backs.

Sensitivity and specificity

The outcome of unconsciousness of the indicator 'corneal reflex' is absence of the corneal reflex. Therefore, the specificity is the percentage of unconscious animals showing no corneal reflex after neck cutting, out of all animals truly unconscious. This was estimated by questionnaire 2 respondents to be 99 % (*n* = 7). The sensitivity is calculated as the percentage of conscious animals observed to show positive corneal reflex, out of all truly conscious animals. This was estimated to be 82 % (*n* = 8).

3.5.1.7. Response to threatening movement

Description

Conscious animals respond either by blinking or attempting to move away from threatening movements (or clapping) of hands close to the eye. This fear response will be abolished in unconscious animals and therefore **response to visually threatening movements** can be used to check unconsciousness.

Feasibility

From questionnaire 2, response to threaten was considered (*n* = 2) to be difficult to assess. Neither of the experts considers it easy to assess. The main reason might be the fact that the head is restrained and the assessment of head movements might not be possible during this time. Other reasons include lack of access to the animal and the fact that the eyes are usually covered with blood, especially when the animals are rotated on to their backs or the limited number of respondents.

Sensitivity and specificity

The outcome of unconsciousness of the indicator 'response to threatening movement' is absence of a response to threatening movement. Therefore, the specificity is the percentage of unconscious animals which do not respond to threaten movement after neck cutting, out of all animals truly unconscious. This was estimated by questionnaire 2 respondents to be 95 % (*n* = 1). The sensitivity is calculated as the percentage of conscious animals responding to threatening movement, out of all truly conscious animals. This was estimated to be 100 % (*n* = 1).

3.5.1.8. Response to nose prick or ear pinch

Description

Unconsciousness following slaughter without stunning can be determined from the **abolition of response to a painful stimulus such as nose prick** to the muzzle (area between external nostrils) or **ear pinch** with a sharp instrument. Unconscious animals will not show a positive response to a painful stimulus.

Feasibility

From questionnaire 2, the experts reported (n = 4) that response to nose prick or ear pinch is either normal (50 %) or difficult (50 %) to assess. The main reason might be that the head is restrained and the assessment of head movements might not be possible on the restrained animal. Other reasons include lack of access to the head of the animal, which may also be covered with blood, especially when the animals are rotated on to their backs.

Sensitivity and specificity

The outcome of unconsciousness of the indicator 'response to nose prick or ear pinch' is the absence of response to nose prick or ear pinch. Therefore, the specificity is the percentage of unconscious animals which do not respond to nose prick or ear pinch after neck cutting, out of all animals truly unconscious. This was estimated by questionnaire 2 respondents to be 97 % (n = 2). The sensitivity is calculated as the number of conscious animals responding to nose prick or ear pinch, out of all truly conscious animals. This was estimated to be 86 % (n = 3).

A summary of the information on indicator sensitivity, specificity and feasibility collected from questionnaire 2 is presented in Table 8.

Table 8: Summary of the data from questionnaire 2 regarding sensitivity, specificity and feasibility of indicators applied prior to release from restraint during slaughter without stunning

Indicators to be used to detect unconsciousness (prior to release to restraint)	**Outcomes of unconsciousness**	**Sensitivity (%)**	**Data (without uncertainty) No of respondents Average (0, 50th and 80th percentiles)**	**Specificity (%)**	**Data (without uncertainty) No of respondents Average (0, 50th and 80th percentiles)**	**Feasibility (from questionnaire 2)**
Posture	Permanent loss of posture	89	9 87 50;95;100	99	6 97 80;100;100	-0.17
Muscle tone	Loss of muscle tone	96	3 95 90;95;100	100	3 100 100;100;100	0.00
Breathing	Cessation of breathing	97	9 94 60;100;100	92	9 90 20;100;100	0,11
Pupillary reflex	Absence of reflex	100	2 100 100;-;100	98	2 98 95;-;100	-0.50
Palpebral reflex	Absence of reflex	94	5 90 60;100;100	99	4 99 95;100;100	-0.33
Corneal reflex	Absence of reflex	82	8 83 60;100;100	99	7 99 95;100;100	-0.44
Response to threaten	Absence of response	100	1 n.a.	95	1 n.a.	-1.00
Responses to nose prick or ear pinch	Absence of response	86	3 80 50;90;100	97	2 98 95;-;100	-0,50

n.a., not applicable as only one respondent provided data

Please note that the scores presented in Table 8 were the opinion of a small number of people (for some indicators n < 5, as indicated in the above paragraphs) engaged in slaughter without stunning.

3.5.2. Indicators prior to dressing

This list of indicators is intended for use before the carcass dressing begins.

3.5.2.1. Pupil size

Description

Dilated pupils (midriasis) is an indicator of the onset of brain death (outcome of death), the assessment of which requires close examination of the eyes.

Feasibility

From questionnaire 2, the majority of the experts reported ($n = 7$) that dilated pupils are either easy (43 %) or normal (29 %) to assess. Once the animal is released from the restraint, the accessibility to the eye allows assessment of the pupils.

Sensitivity and specificity

The outcome of death of the indicator 'pupil size-' is dilated pupils. Therefore, the specificity is the percentage of dead animals which show dilated pupils, out of all truly dead animals. This was estimated by questionnaire 2 respondents to be 100 % ($n = 5$). The sensitivity is calculated as the percentage of live animals observed without dilated pupils, out of all truly alive animals. This was estimated to be 99 % ($n = 5$).

3.5.2.2. Muscle tone

Description

Complete and irreversible loss of muscle tone leads to **relaxed body of the animal**, which can be recognised from the limp carcass, is an outcome of death.

Feasibility

From questionnaire 2, muscle tone was considered ($n = 6$) to be easy or normal to assess by 56 % and 33 %, respectively, of experts. After the animal is released from the restraint, the relaxed body should be easy to assess.

Sensitivity and specificity

The outcome of death of the indicator 'muscle tone' is a relaxed body. Therefore, the specificity is the percentage of dead animals which show relaxed body, out of all truly dead animals. This was estimated by questionnaire 2 respondents to be 100 % ($n = 3$). The sensitivity is calculated as the percentage of live animals showing certain maintenance of muscle tone, out of all truly live animals. This was estimated to be 93 % ($n = 3$).

3.5.2.3. Bleeding

Description

Slaughter leads eventually to cessation of bleeding, with only minor dripping, from the neck cut wound, and therefore **end of bleeding** in both carotid arteries and jugular veins can be used as an outcome of death. However, formation of aneurysm and occlusion of the carotid artery (known as carotid artery ballooning) may prevent blood flow from the neck cut wound, and this should not be mistaken for end of bleeding. Partial aneurysm and occlusion of carotid artery may be visible and recognised from blood squirting out.

Feasibility

From questionnaire 2, bleeding was rated ($n = 7$) as easy to assess by 71 % and as normal to assess by 29 %.

Sensitivity and specificity

The outcome of death of the indicator ‘bleeding’ is the end of bleeding. Therefore, the specificity is the percentage of dead animals in which bleeding has ended after neck cutting, out of all truly dead animals. This was estimated by questionnaire 2 respondents to be 97 % ($n = 5$). The sensitivity is calculated as the percentage of live animals bleeding, out of all truly live animals. This was estimated to be 98 % ($n = 6$).

3.5.2.4. Cardiac activity

Description

Onset of death leads to permanent **absence of cardiac activity** (absence of heart beat), which can be ascertained using a stethoscope.

Feasibility

From questionnaire 2, cardiac activity was rated ($n = 3$) as difficult to assess by 100 % of experts. Cardiac activity is normally assessed with a stethoscope, which might not be available in slaughterhouses.

Sensitivity and specificity

The outcome of death of the indicator ‘cardiac activity’ is the absence of a heart beat. Therefore, the specificity is the percentage of dead animals without cardiac activity after neck cutting, out of all truly dead animals. This was estimated by questionnaire 2 respondents to be 100 % ($n = 2$). The sensitivity is calculated as the percentage of live animals showing presence of heart beat out of all truly alive animals. This was estimated to be 100 % ($n = 2$).

3.5.2.5. Pulse rate

Description

Onset of death leads to permanent loss of pulse. Pulse can be ascertained physically by pressing the (uncut) arteries in an extremity (e.g. the femoral or coccygeal artery), and the absence of a pulse can be used to confirm death in animals.

Feasibility

From questionnaire 2, pulse rate was considered ($n = 2$) to be difficult to assess by all experts. The reason for this high rating is unclear, but it may be experts are not familiar with the methodology to assess pulse rate in bovines.

Sensitivity and specificity

The outcome of death of the indicator ‘pulse rate’ is the absence of pulse. Therefore, the specificity is the percentage of dead animals without a pulse, out of all truly dead animals. This was estimated by questionnaire 2 respondents to be 100 % ($n = 3$). The sensitivity is calculated as the percentage of live animals showing a positive pulse rate, out of all live animals. This was estimated to be 100 % ($n = 5$).

A summary of the information on indicator sensitivity, specificity and feasibility collected from questionnaire 2 and the systematic literature review is presented in Table 9.

Table 9: Summary of the data from questionnaire 2 and the systematic review regarding sensitivity, specificity and feasibility of indicators of death applied prior to dressing during slaughter without stunning

Indicators to be used (prior to carcase dressing)	Outcomes of death	Sensitivity (%)	Data (without uncertainty) No of respondents Average (20th, 50th and 80th percentiles)	Specificity (%)	Data (without uncertainty) No of respondents Average (20th, 50th and 80th percentiles	Feasibility
Pupil size	Dilated pupils	99	5 99 95;100;100	100	5 100 100;100;100	0.14
Muscle tone	Relaxed body	93	3 92 75;100;100	100	3 100 100;100;100	0.44
Bleeding	End of bleeding	98	6 98 90;100;100	97	5 96 80;100;100	0.71
Cardiac activity	Absence of cardiac activity	100	2 100 100;-;100	100	2 100 100;-;100	-1.00
Pulse rate	Absence of pulse rate	100	5 100 100;100;100	100	3 100 100;100;100	-1.00

Please note that the scores presented in Table 9 were the opinion of a small number of people (for some indicators $n = 2$, as indicated in the above paragraphs) engaged in slaughter without stunning.

4. Discussion

4.1. Introduction

As previously described, this scientific opinion proposes welfare indicators to be used for monitoring during the slaughtering process of bovine animals. In order to allow effective monitoring, the animals must be able to express behaviours and reflexes associated with consciousness. Consequently, procedures, processes or treatments that could mask the expression of such behaviours (such as electrical immobilisation or electrical stimulation) should not be used prior to confirmation of death in animals. Welfare indicators to be included in a 'toolbox' will be selected based on their sensitivity, specificity and feasibility, as derived from various activities that were carried out for the development of the opinion. Owing to the lack of scientific publications involving simultaneous assessment of EEG indicators of unconsciousness and welfare indicators (such as physical reactions and reflexes), the systematic literature review was not very productive and, therefore, much of the information for the selection of the indicators comes from questionnaire 2, which was specially aimed at obtaining estimates of the sensitivity, specificity and feasibility of the indicators. The indicators proposed in the toolboxes were selected based on these findings and on an expert consultation process (public consultation and technical meeting with experts from interested parties on 3 September 2013). Similarly, the model proposed for the sampling protocols was discussed with interested parties. The description of indicators in sections 3.4 and 3.5 also contains some basic information about elicitation of reflexes and responses and how to use the indicators. This is particularly relevant for indicators that warrant evoking a response from the animals (e.g. the corneal reflex). A short description of the physiology and elicitation of the indicators or evoking a conscious response is also presented in the glossary.

Indicators additional to those recommended in the toolboxes can also be used if considered necessary. Although the questionnaire was structured and presented to the respondents in such a way as to avoid confusion between sensitivity, specificity and feasibility, close examination of the data revealed that sensitivity ratings given to some of the indicators were influenced by the feasibility of checking under the two different scenarios, i.e. captive bolt stunning and slaughter without stunning. For example, eliciting and/or recognising corneal and palpebral reflexes or response to nose prick is not easy in the case of slaughter without stunning, especially when the animals are rotated on to their backs, because of the blood pouring over the head of the animal. Owing to this reduced feasibility, the sensitivity and specificity scores given to these indicators were lower than those was given for monitoring during bleeding following captive bolt stunning situation. Evidently, the feasibility scores given to some indicators for checking after stunning, at neck cutting/sticking or during bleeding under captive bolt stunning situation were also different because of lack of access to the carcass. For example, the feasibility score for response to nose prick was significantly reduced for monitoring during bleeding when compared with after stunning.

It should also be noted that the sample size, i.e. number of respondents to the questionnaire, was small and respondents were mainly from small to medium-sized FBOs; nevertheless, the questionnaire indicates the existing knowledge, understanding and skill levels.

The outcomes of questionnaire 2 and the systematic review were also discussed with external hearing experts at a meeting held on 3 September 2013. During the meeting, consensus was achieved on a set of recommended indicators to be included for each toolbox. Furthermore, for each toolbox, additional indicators which can be used, but which have lower sensitivity or feasibility, and are therefore not sufficient by themselves, were identified. The external experts advised that provision of a limited number of recommended indicators and a few more additional indicators was confusing and too prescriptive. In addition, they argued that skill levels in slaughterhouses and the feasibility of assessing the indicators may vary from slaughterhouse to slaughterhouse and therefore the toolbox should include more indicators. The external experts also felt that provision of indicators alone is not helpful in decision making and therefore a flow chart should be considered.

The outcomes of questionnaire 2 and discussion with hearing experts suggested that the low sensitivity and specificity ratings given to some outcomes of consciousness could explain why the overall practice is to look at outcomes of unconsciousness, which is the expected outcome of stunning, rather than detection of consciousness as a poor welfare outcome. Misconceptions with regard to the physiological basis of indicators were also inferred. These misconceptions need to be eliminated to harmonise welfare monitoring in slaughterhouses. It is also suggested that the sensitivity and specificity of these indicators would improve as people acquire relevant knowledge, skill and experience in assessing them. The feasibility scores reported in this opinion are also based upon limitations of the existing infrastructure, which is not necessarily designed and constructed with welfare monitoring as a priority. Therefore, it is suggested that the feasibility of monitoring these indicators would also improve if welfare monitoring were taken into consideration during the design, layout and construction of a new slaughterhouse, or following a structural change to existing slaughterhouses.

The monitoring procedures are intended for use by the FBO in order to prevent negative welfare outcomes for the animals. The FBO, as a licence holder of a slaughterhouse, and employees with responsibility for animal welfare, including those designated as animal welfare officers, should undergo proper training and assessment of competence in welfare monitoring before licences are granted. For this to occur, any training, assessment and certification programmes implemented by the Member States should include welfare monitoring, and the contents of such education/training courses should be harmonised. Within the scope of Regulation (EC) 1099/2009, standard operating procedures should be implemented by the FBO and Member States/Competent Authorities should develop guides to good practice. These instruments should include welfare monitoring protocols/procedures for all key stages.

In addition, the regulation requires that personnel carrying out stunning or bleeding have a certificate of competence and the awarding of such certificates should also be contingent on the ability to monitor animal welfare.

4.2. Monitoring procedures for captive bolt stunning

4.2.1. Combination of selected indicators (the 'toolboxes')

For the creation of the toolboxes of indicators to be used in the monitoring procedures, indicators and their outcomes were selected by the Working Group members based on their knowledge regarding the validity, feasibility and sensitivity of the indicator. The specificity is not relevant for the toolbox considered to address potential welfare issues using consciousness as outcome (see section 2.1.2).

Indicators with high sensitivity and feasibility ratings in the questionnaire were selected for the toolbox. Some additional indicators that were given relatively lower ratings for sensitivity or feasibility were also included because the hearing experts and the Working Group thought that some of these indicators, such as eye movement in cattle stunned with captive bolt, might have a good feasibility (ease of use) in slaughterhouses. The experts of the Working Group also agreed that indicators given low sensitivity and specificity at present by the respondents to the questionnaire might have potential for improvement in the future through education, training and assessment of personnel with responsibility for monitoring and ensuring welfare at slaughter (i.e. award of the Certificate of Competence). Similarly, indicators with low feasibility at present could be improved by changes in the design and layout or changes to existing practice. It was also thought that the toolbox should contain practical guidance with regard to recognition of consciousness and the decision-making process.

Indicators can be used either in parallel or in series. If two or more indicators are used in series, the second indicator is checked conditional on the outcome of the first indicator applied; if two or more indicators are used in parallel, they are performed simultaneously and therefore the animal is considered conscious when at least one of the indicators is positive.

For the purpose of detecting conscious animals in the slaughter line, indicators should be used in parallel. Indicators from the toolbox must be checked simultaneously on each sampled animal. To rule out consciousness, it is necessary that none of the indicators selected from the toolbox shows the outcome of consciousness. In practice, however, action may already have been taken, if there is evidence of consciousness, before all indicators have been checked.

When applying more than one indicator, it seems reasonable to expect an increase in the probability of detecting conscious animals, i.e. higher overall sensitivity of the monitoring protocol. If the checked indicators will show their outcomes independent of each other, then the overall sensitivity indeed increases. However, this possible increase in sensitivity would be less if the outcomes of the indicators are correlated, e.g. as a result of common physiological grounding or the checking procedure itself. The exact quantification of this correlation is not yet possible owing to a lack of scientific information. But it can be shown that the joint sensitivity of two or more indicators is at least equal to the highest sensitivity of either or any (Gardner et al., 2000). Therefore, and in the absence of quantified correlation between indicator outcomes, it is recommended that more than one indicator be used for the monitoring, but to consider the highest sensitivity of the selected indicators when planning the required sample size. This approach may lead to an oversampling, which, on the other hand, is in line with the precautionary principle needed to protect the welfare of animals.

Toolbox for key stage 1 (Toolbox 1 = immediately after stunning)

This opinion recommends the following indicators (and their outcomes of consciousness) for inclusion in the toolbox at key stage 1: posture, breathing, tonic seizure, corneal reflex (these are presented above the dashed line in the flow chart). Additional indicators—muscle tone, eye movements and vocalisation—are also proposed (these are presented below the dashed line in the flow chart), but their sensitivity or feasibility is lower and they should not be relied upon alone.

The reasons for this approach are presented in the following paragraphs.

Recommended indicators (above the dashed line in the flow chart)

Posture

The respondents rated the sensitivity of the outcome of unconsciousness ‘immediate collapse’ very low. Indeed, this rating was considered reasonable, as immediate collapse may occur in animals which are not rendered unconscious following captive bolt stunning, for example because of incorrect shooting position (see section 3.4). However, the experts felt that immediate and permanent collapse is a reliable outcome of unconsciousness and is routinely used in slaughterhouses to recognise effective captive bolt stunning. As a precaution, further checks should be performed during removal of animals from the stun box to ensure that the collapse was indeed permanent and the animal presents no outcomes of consciousness (e.g. rotation of the eyes or rhythmic breathing). For the purpose of the monitoring procedure, the appropriate outcome for detecting conscious animals would be the ‘attempt to regain posture’. Indeed, attempt to regain posture can be used to recognise consciousness after stunning and during shackling, hoisting, sticking and bleeding.

Breathing

This indicator is feasible and sensitive according to questionnaire 2 and can be used after the animals have been removed from the stun box, i.e. during shackling, hoisting, sticking and bleeding.

Tonic seizures

This indicator has a high feasibility and sensitivity and can be used especially in key stage 1. Captive bolt-stunned animals collapse immediately after shooting in the stunning box and they can be seen only after they are rolled out of the stun box for shackling and hoisting. Tonic seizure may sometimes end before the animals are rolled out of the stun box, especially when there is a long delay between shooting the animal and releasing it from the stun box.

Corneal or palpebral reflex

In questionnaire 2, corneal and palpebral reflexes were considered respectively as highly and moderately sensitive. However, it was suggested during working group discussions that people performing checks usually touch the whole eye intending to provoke blinking in conscious animals, may not always make distinction between corneal and palpebral reflexes. Therefore, these two eye reflexes are to be used in combination.

Additional indicators (below the dashed line in the flow chart)

Muscle tone

This indicator is sufficiently feasible and sensitive according to questionnaire 2. Recovery of different degrees of muscle tone may be manifested as foreleg flexion, hind leg kicking, stiff ears, head-righting reflex, arching of the back, and these signs can be used to recognise consciousness.

Eye movements

This was considered a feasible indicator, although not very sensitive. However, the ‘presence of eye movement’ should be considered a ‘warning signal’ warranting further investigation.

Vocalisations

This was considered a very feasible indicator, although not very sensitive. However, the ‘presence of vocalisations’ such as grunting, bellowing or mooing, should be taken seriously and warrants intervention (re-stunning).

Indicators not considered in the flow chart

The following indicators were not considered in the flow chart because of their low sensitivity or feasibility, e.g. limited or no access to the animal (see section 3.4): response to nose prick or ear pinch, body movements and pupillary reflex.

Toolbox for key stage 2 (Toolbox 2 = during neck cutting or sticking)

This opinion recommends that the following indicators be included in the toolbox at key stage 2: body movements, muscle tone and breathing (these are presented above the dashed line in the flow chart). In addition, eye movements, the corneal reflex and spontaneous blinking may also be used (these are presented above the dashed line in the flow chart).

The reasons for this are as follows.

Recommended indicators (above the dashed line in the flow chart)

Body movements

The data from questionnaire 2 suggest that 'body movements' are sufficiently sensitive and very feasible to score. While the absence of body movements in response to sticking does not necessarily mean that the animal is unconscious, the 'presence of body movements' in response to sticking should be considered a 'warning signal' warranting further investigation.

Muscle tone

The data from questionnaire 2 suggest that the assessment of 'muscle tone' is sufficiently sensitive and feasible to score. According to the experts' opinion, recovery of different degrees of muscle tone may be manifested as foreleg flexion, hind leg kicking, stiff ears, head-righting reflex and arching of the back, and these signs can be used to recognise consciousness. Therefore, muscle tone was included in the toolbox.

Breathing

The data from questionnaire 2 suggest that the assessment of 'breathing' is sufficiently sensitive and feasible to score.

Additional indicators (below the dashed line in the flow chart)

Eye movements

This indicator was rated lowly feasible and sensitive. However, experts' opinion suggested that 'eye movements' can be used to recognise consciousness after captive bolt stunning and during shackling, hoisting, sticking and bleeding.

Corneal or palpebral reflex

In questionnaire 2, corneal and palpebral reflexes were considered respectively as highly and moderately sensitive. However, it was suggested during working group discussions that people performing checks usually touch the whole eye intending to provoke blinking in conscious animals, may not always make distinction between corneal and palpebral reflexes. Therefore, these two eye reflexes are to be used in combination. The feasibility of both indicators is reduced in comparison to key stage 1 when the animal's head is covered in blood following sticking. However, the presence of the corneal or palpebral reflex should be used as a warning signal to check for other outcomes of consciousness.

Spontaneous blinking

The data from questionnaire 2 suggest the indicator of blinking to be sufficiently sensitive and feasible to assess consciousness during key stage 2. While the absence of spontaneous blinking does not necessarily mean that the animal is unconscious, the ‘presence of spontaneous blinking’ can be used to recognise consciousness after stunning and during shackling, hoisting, sticking and bleeding.

<u>Indicators not considered in the flow chart</u>

The following indicators were not included in the flow chart because of their low sensitivity or feasibility ratings, because access to the animal is limited or absent (see section 3.4): posture, tonic seizures, response to nose prick or ear pinch, body movements, vocalisation and the pupillary reflex.

It should be noted that several respondents rated as high the feasibility of ‘tonic seizures’ during neck cutting and bleeding. Tonic seizures are expected to occur immediately after stunning and last for only seconds, and therefore end before neck cutting or sticking is performed. However, tonic seizures could still be present at neck cutting if the stun-to-stick interval is short. Animals may show involuntary movements of the legs during neck cutting or sticking and bleeding, and these may be interpreted as being part of the seizures. For this reason, it was decided not to consider tonic seizure as an indicator for the toolbox at neck cutting or during bleeding (i.e. not applicable).

Toolbox for key stage 3 (Toolbox 3 = during bleeding).

This opinion proposes the following indicators to be included in the toolbox at key stage 3: muscle tone, breathing and spontaneous blinking.

The reasons for this are as follows.

<u>Recommended indicators</u>

Muscle tone

The data from questionnaire 2 suggest the indicator of muscle tone to be relatively sensitive and feasible to score during this key stage. Recovery of different degrees of muscle tone may be manifested as foreleg flexion, hind leg kicking, stiff ears, head-righting reflex or arching of the back, and these signs can be used to recognise consciousness, especially during bleeding.

Breathing

The data from questionnaire 2 suggest the indicator of breathing to be sufficiently sensitive and feasible to score during this key stage.

Spontaneous blinking

The data from questionnaire 2 suggest the indicator of blinking to be sufficiently sensitive and feasible to score during this key stage. While the absence of spontaneous blinking does not necessarily mean that the animal is unconscious, the ‘presence of spontaneous blinking’ can be used to recognise consciousness during bleeding.

<u>Indicators not considered in the flow chart</u>

The following indicators were not recommended and not considered in the flow chart because of their low performance or their low feasibility when access to the animal is limited (see section 3.4): tonic seizure, response to nose prick or ear pinch, body movements, vocalisation, eye movements, palpebral reflex, corneal reflex and pupillary reflex.

4.2.2. Flow chart for the use of the toolbox indicators at slaughter with captive bolt stunning

A flow chart was designed to support the understanding of the use of the indicators and is shown in Figure 5. Please refer to the section 3.2 for the definitions and selection process of the indicators and refer to section 3.4 and Table 7 for the sensitivity of each indicator (that is used to calculate the sample size).

The flow chart in Figure 5 illustrates this opinion's recommendations regarding the three key stages of monitoring, the recommended outcomes of consciousness or unconsciousness and the course of action to be taken when outcomes of consciousness are detected in cattle following captive bolt stunning. Following the stun, and prior to shackling (key stage 1), the four indicators listed above the dashed line in the blue Toolbox 1 are recommended to be used to recognise consciousness. The indicators below the dashed line also can be used to check for signs of consciousness, but their sensitivity is low and they should not be relied upon on their own. If the animal shows any of the signs of consciousness (red box), then appropriate intervention should be applied.

If all indicators suggest that the animal is unconscious (green box), then the animal can be shackled and bled out by a neck cut or chest stick. In the Toolbox 2, three recommended indicators are presented above the dashed line, and these can be used to check for consciousness at key stage 2. There are additional indicators below the dashed line in this Toolbox 2 which may also be used to check for outcomes of consciousness, but with low sensitivity. If the animal shows any of the outcomes of consciousness (red box), then appropriate intervention should be applied.

If all the indicators suggest unconsciousness (green box), then the animal should continue to be checked during bleeding (key stage 3). The blue Toolbox 3 recommends indicators to be used to check for consciousness. If any one outcome of these indicators suggests consciousness (red box), then appropriate intervention should be applied. If the indicators presented in the Toolbox 3 suggest unconsciousness (green box), then it can be concluded there is no risk of consciousness being regained.

Out of the recommended indicators above the dashed line, a minimum of two indicators relevant to each key stage should be employed for an effective monitoring of the process.

Please note that for those indicators which rely on animal to manifest certain behaviour suggestive of consciousness (e.g. spontaneous blinking, vocalisations) their outcomes of unconsciousness are presented in grey shade to recall the limited predictive value of the indicator i.e. the percentage of non-vocalising animals that are truly unconscious out of all non-vocalising animals). Nevertheless, the outcome of consciousness suggests that the animal is conscious and is a "warning signal" requiring an intervention.

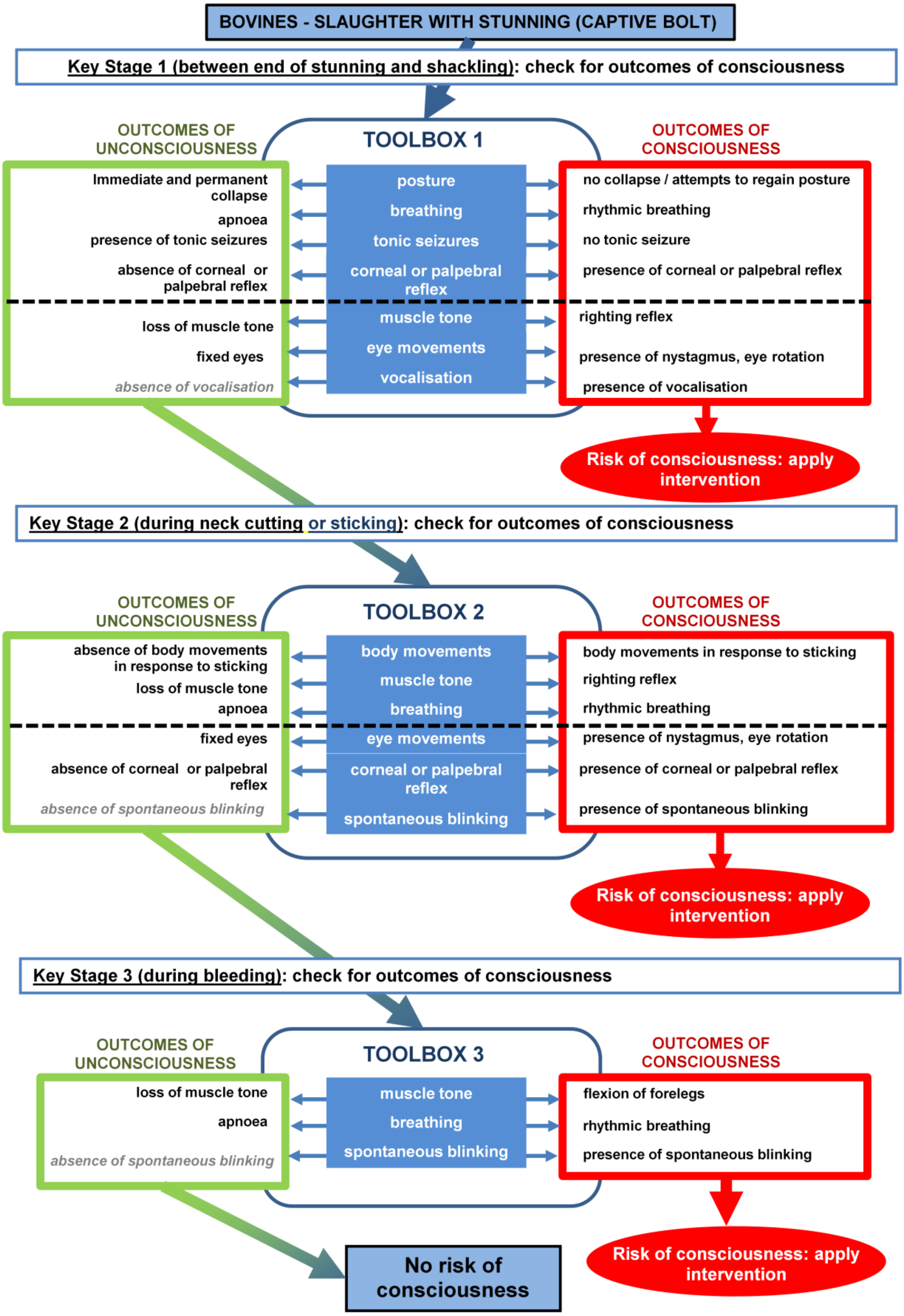


Figure 5: Toolbox of indicators that are considered suitable to be used for detection of conscious animals at each key stage of the procedure of captive bolt stunning in bovines

4.2.3. Sampling protocol for captive bolt stunning

Independent of the sampling protocol specified in section 2.3.2. and discussed below, but in line with the duties of the personnel, who should process only unconscious animals, all animals (SF 100 %) should be monitored to prevent poor welfare outcomes. The indicators suggested in the flow chart are aimed at achieving effective monitoring of welfare of the animals by all personnel involved in stunning and slaughter.

4.2.3.1. Risk factors and welfare consequences

The final welfare consequence of failed captive bolt stunning is the risk of conscious or not fully unconscious animals being shackled, stuck or dressed. This risk needs to be reduced to zero, by ensuring proper stunning routines and monitoring of stun efficacy.

In order to develop a monitoring protocol, the mandate from the Commission requests EFSA to estimate the optimal frequency with which animals should be checked for signs of consciousness following stunning.

This frequency should take into account risk factors associated with the stunning procedure.

The most common risk factors involved in the welfare of animals during slaughter are listed in Table 10. They have been linked to two categories: those risk factors that affect the quality of the stun and those that affect the quality of the assessment.

The two types of risk factors have a different effect on the sampling protocol.

Risk factors that reduce the quality of the stun

When the quality of the stun is reduced, the probability of an animal not being properly stunned increases. This will increase the number of conscious animals which are presented to the operator for checking, i.e. increase the failure rate. The model-based sampling procedure developed in Section 2.3 is designed to detect any increase in this proportion of mis-stunned animals: in particular, the system will detect at least one conscious animal as soon as the overall proportion of poorly stunned animals exceeds the set failure rate. Therefore, in the case of risk factors affecting the quality of the stun, the frequency of sampling does not have to be increased even though the number of animals that are mis-stunned increases. These risk factors do not necessitate any change in sampling fraction.

Risk factors that reduce the sensitivity of the indicators used

Factors reducing the effectiveness of the assessment of consciousness will increase the likelihood that conscious animals are processed as if they were unconscious. This is, of course, an undesirable situation from an animal welfare point of view. If we deal with the indicators as if they were a diagnostic test, the ‘effectiveness’ of an indicator is expressed by the sensitivity, i.e. the probability of correctly classifying a truly conscious animal as conscious. It is intuitive that the lower this probability (i.e. the sensitivity of the indicator), the greater the number of animals that have to be tested in order to achieve a consistent level of confidence. This relationship is quantified through the model developed in Chapter 2.

The quantification of these sensitivity values is based on the knowledge and experience of a pool of stakeholders who were requested to fill in questionnaire 2 (see section 3.3). Therefore, the resulting figures have to be referred to as ‘regular’ or ‘average’ for the situation. As a consequence, it is plausible to assume that under certain circumstances or ‘risk factors’ (e.g. the employment of new personnel) the same indicator may perform worse than under regular circumstances. Quantitatively speaking, when dealing with these different conditions, the sensitivity reference values may no longer hold; thus, the sample size required under these circumstances will be larger. These risk factors will therefore affect the monitoring procedure, because they alter the sensitivity of the indicator.

Table 10: Risk factors to animal welfare associated with captive bolt stunning of bovines

Component	Risk factor	Risk of poor stunning (a)	Risk of poor assessment (a)
Staff	Competence	√	√
	Experience	√	√
	Fatigue	√	√
Equipment	maintenance	√	
	Features (e.g. gun, cartridge, restraining device)	√	
	Presence of records of maintenance (e.g. cleaning)	√	
Records of the checks	Conformity in the past	√	√
Animals	Body weight	√	√
	Category/breed/temperament	√	√
Establishment	Line speed	√	√

(a): The choice of risk category is based on expert opinion only.

4.2.3.2. Different scenarios for the sampling protocols

The risk factors described in the previous paragraph may require changes to the sampling protocol applied in the slaughterhouse. Three levels of sampling can be identified: standard, reinforced and light (also referred to in the literature as normal, tightened and reduced inspections).

'Standard' sampling protocol

The standard operating procedure for slaughter of cattle will involve a sampling fraction of 100 % by slaughterhouse personnel, as the operators check each animal for indicators of consciousness immediately after stunning, before sticking and during bleeding. In addition, the animal welfare officer will sample a fraction of all animals to monitor the effectiveness of the process, and will correct the operator or other aspects of the stunning process if necessary. The fraction sampled by the welfare officer can be calculated by the model, and is dependent on the indicator sensitivity, the slaughtered population, the maximum allowed threshold FR and the required accuracy, as described previously.

The larger the chosen slaughter population, or the higher the threshold FR, the lower the resulting sampling fraction will be. This means that the number of animals between two consecutively tested animals becomes larger. For example, if we take a required accuracy of 95 %, and an indicator with a sensitivity of 90 %, then the following calculation illustrates the effects of a risk manager's decision regarding threshold FR and slaughter population. Given a slaughter population of number of animals killed on one day (e.g. 500 animals), and a threshold failure rate of 0.01, the sampling fraction will be 50 %. Therefore, one in every two animals will need to be monitored. However, if the slaughter population is set at one working week (at the same daily throughput, so 2 500 animals), then the sampling fraction will be 13 %: so one in every eight animals. An appropriate decision on the criterion for defining a slaughter population and threshold FR would therefore help to achieve the requirements of the legislation on animal welfare at slaughter.

It goes without saying that the sampling protocol itself should not be a reason to delay the procedure. When slaughterhouse personnel identify a mis-stunned animal, they should take immediate remedial action. Later, the personnel have to identify the reason for the poor stun and take remedial action. They then inform the FBO or animal welfare officer.

An animal welfare officer who identifies a mis-stunned animal during execution of his/her sampling procedure should take remedial action and initiate the reinforced sampling protocol.

‘Reinforced’ sampling protocol

If one of the above-mentioned risk factors is present, which suggests a reduction in the sensitivity of the indicator applied by the personnel, the welfare officer will need to reinforce the sampling. This can be done by concentrating the sampling efforts in a shorter time following the introduction of the risk factor, until the risk is identified and rectified. The degree to which the sampling needs to be increased is determined by the incurred reduction in indicator sensitivity. However, because the reduction in indicator sensitivity is not available, a pragmatic approach is required. This is to test all animals during a period represented by one-tenth of the slaughtered population. For example, if the slaughtered population as referred to in the standard sampling protocol was set to 200 animals. Then for the time until the next 20 animals are processed, i.e. one-tenth of the slaughter population, all animals have to be retested by the back-up sampling.

‘Light’ sampling protocol

There are no circumstances under which the sampling frequency (SF) of the welfare officer can be relaxed, as a reduction in the SF will immediately reduce the accuracy with which a given excess threshold failure rate may be detected by the monitoring protocol (the other factors of the model, slaughtered population and test sensitivity, being unchanged).

4.3. Monitoring procedures for slaughter without stunning

4.3.1. Combination of selected indicators (the ‘toolboxes’)

As explained in section 2.1.2.2., since, in the case of slaughter without stunning, unconsciousness and death are induced gradually, indicators checking the state of unconsciousness and death were selected based on their specificity to detect unconscious animals out of all unconscious animals and preferably based on their sensitivity to detect animals still truly conscious as conscious. The sensitivity of the indicators was considered together with the feasibility of each stage. Here, the specificity—the number of unconscious animals detected out of all unconscious animals—would be less relevant for the purpose of monitoring welfare as no further processing can occur as long the outcome of the checked indicator suggests consciousness, regardless whether that outcome is true or false.

It was evident from the literature that aneurism and occlusion of carotid artery, known as ballooning, is an occurrence during slaughter without stunning of bovines and strongly argued by the experts that the onset of such an event within few seconds of neck cutting should be recognised early and considered as an ‘ALARM’ warranting immediate intervention. Therefore, ballooning was included as an indicator of prolonged consciousness or delayed onset of unconsciousness deserving a separate box in the toolbox.

As explained in section 4.2.1, with the purpose of detecting unconscious and dead animals in the slaughter line, indicators can be used in parallel.

Toolbox for indicators prior to release from restraint for slaughter without stunning (Toolbox 4)

This opinion recommends the following indicators for inclusion in the toolbox: breathing and muscle tone. Additional indicators—posture and corneal or palpebral reflex—are also proposed, but they should not be relied upon alone.

The reasons for this are as follows.

Recommended indicators (above the dashed line in the flow chart)

Breathing

Breathing is considered a highly sensitive and reasonably feasible indicator according to questionnaire 2 and the ‘absence of breathing’ can be used to confirm unconsciousness prior to release from restraint.

Muscle tone

Muscle tone is considered a highly sensitive and reasonably feasible indicator according to questionnaire 2. Moreover, 'loss of muscle tone' can be used to confirm unconsciousness prior to release from restraint.

Additional indicators (below the dashed line in the flow chart)

Posture

Posture is considered a reasonably specific and reasonably feasible indicator but has limited sensitivity.

Corneal or palpebral reflex

In questionnaire 2, corneal and palpebral reflexes were considered respectively as highly and moderately sensitive. However, it was suggested during working group discussions that people performing checks usually touch the whole eye intending to provoke blinking in conscious animals, may not always make distinction between corneal and palpebral reflexes. Therefore, these two eye reflexes are to be used in combination. It was also evident that it is not easy to check them separately when the animal's head is covered in blood, especially if the animal is rotated on to its back. However, the presence of the corneal or palpebral reflex should be used as a warning signal to check for other outcomes of consciousness prior to carcass dressing.

Indicators not considered in the flow chart

The following indicators are not recommended and are not considered in the flow chart because of their low performance or their low feasibility (see section 3.5) when access to the animal is limited: papillary reflex, response to threatening movement and responses to nose prick or ear pinch.

Toolbox for indicators prior to dressing for slaughter without stunning (Toolbox 5)

This opinion proposes that the following indicators be included in the toolbox for the assessment of death in slaughter without stunning: end of bleeding, relaxed body, dilated pupils.

The reasons for this are as follows.

Bleeding

Bleeding is sensitive, specific and feasible. Particularly, the end of bleeding is the very specific outcome for the assessment of death.

Muscle Tone
Muscle tone is sensitive and specific. Particular, lack of muscle tone and a fully relaxed body is a highly specific outcome of death.

Pupil size
Pupil size is sensitive, specific and reasonably feasible. Particular, dilated pupils are the highly specific outcome of death.

Indicators not considered in the flowchart

The following indicators were not recommended and not considered to be in the flowchart for their low feasibility (see paragraph 3.5.) due to the limited access to the animal: cardiac activity and pulse rate.

4.3.2. Flow chart for the use of the toolbox indicators for slaughter without stunning

A flow chart was designed to support understanding the use of the indicators.

The flow chart in Figure 6 illustrates this opinion's recommendations regarding the key stages of monitoring, important outcomes of consciousness or unconsciousness and the course of action to be taken when outcomes of consciousness and life are detected in cattle slaughtered without stunning. Following neck cutting and prior to release from restraint, it is recommended that the two indicators listed above the dashed line in the blue Toolbox 4 are recommended to be checked. The indicators below the dashed line may also be checked, but they may become difficult to ascertain under certain conditions (severe restraint and rotation). If the indicators suggest that the animal is still conscious (red box) and bleeding, then the animals should not be released from the restraint. If signs of carotid ballooning are seen, or if bleed-out is slow, then appropriate intervention should be applied.

If all the indicators suggest unconsciousness (green box) and there is no sign of carotid ballooning, then the animal can be released from the restraint. Prior to dressing the indicators in the blue Toolbox 5 should be checked. If all indicators show outcomes presented in the green box, then the animal can be assumed to be dead.

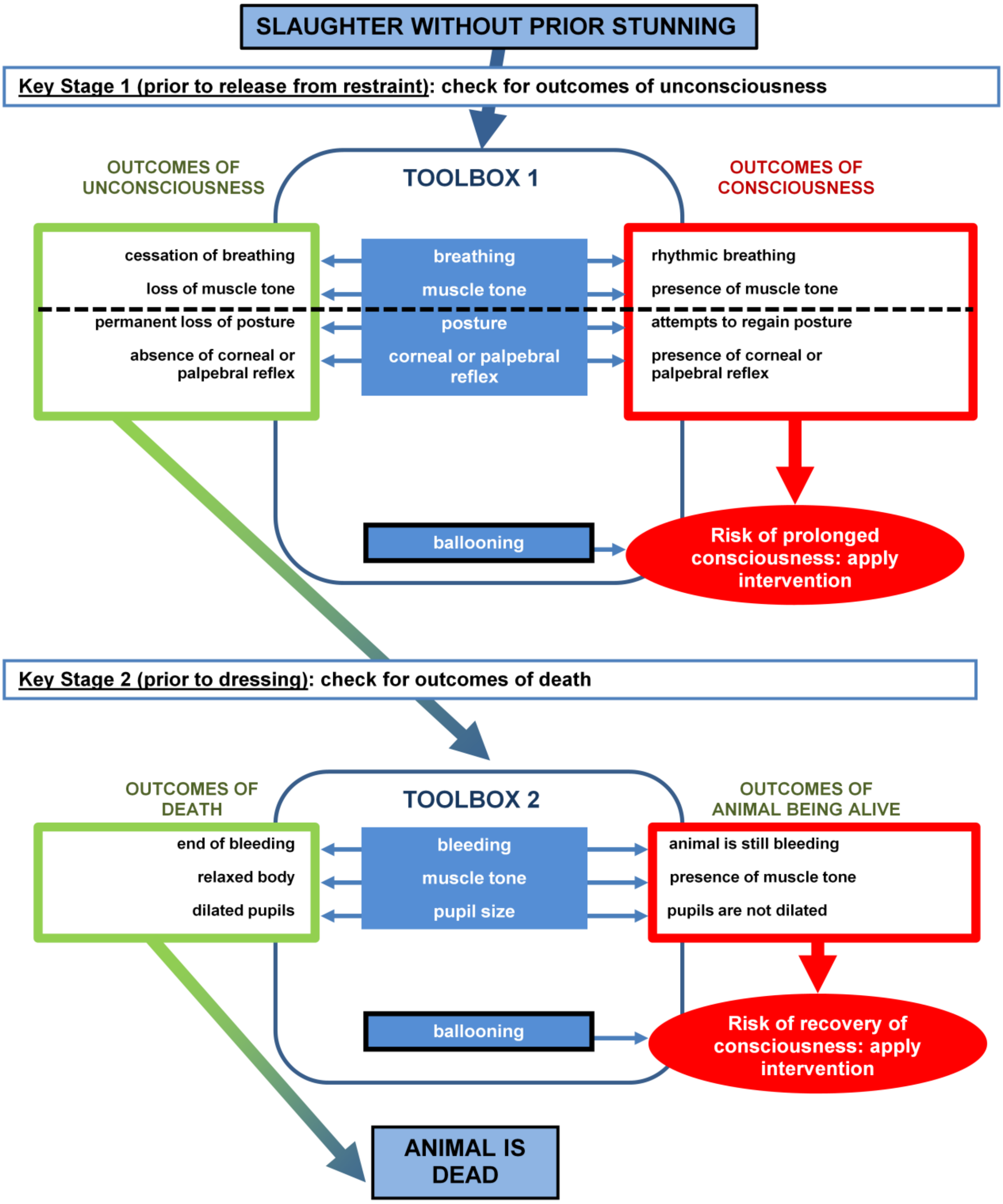


Figure 6: Toolbox of indicators and their outcomes applicable prior to release from restraint and indicators and their outcomes applicable prior to dressing for slaughter without stunning in bovines that are considered suitable to be used for confirmation of animals becoming unconscious and dead as well as detection of animals still conscious or alive. Please refer to section 3.5 for the definitions and selection process of the indicators.

4.3.3. Sampling protocol for slaughter without stunning

According to Regulation (EC) 1099/2009, when cattle are killed without prior stunning, the persons responsible for slaughtering shall carry out systematic checks to ensure that the animals present signs of unconsciousness before being released from restraint and signs of death before undergoing dressing or scalding. Therefore, the personnel responsible for slaughtering should carry out the monitoring in all animals slaughtered without stunning.

4.3.3.1. Risk factors and welfare consequences

The legislation requires the inspection frequency of animals being slaughtered without stunning to be 100 %. Although several aspects of the neck cutting procedure are likely to affect the time to unconsciousness or death, their presence or absence does not affect the frequency of inspection, or any further actions required by slaughter personnel.

Risk factors affecting the quality of neck cutting have been described in the DIALREL report (von Holleben et al., 2010). The restraining device, including both the body and head restrainers, might affect the assessment of unconsciousness. The design of the head restraint must be such that the restraint does not obscure the front of the head and it should also allow good access to the eyes to check the corneal (or palpebral) reflex, and to the trachea to assess breathing.

CONCLUSIONS AND RECOMMENDATIONS

GENERAL CONCLUSIONS

1) From the stakeholder meeting it was apparent that experts currently use several indicators to assess unconsciousness and death in animals. However, no harmonised list of indicators, either species or method specific, is available, nor is there any scientific rationale. This highlights the need to develop a scientifically based set of indicators and monitoring protocols.

2) A systematic literature review found no study that has explicitly reported the sensitivity and specificity of the indicators in unconscious animals—as measured by brain activity using electroencephalogram (EEG). Therefore, there is a scarcity of scientific publications reporting a correlation between unconsciousness or death ascertained by EEG and behavioural and physiological indicators of unconsciousness and death that could be used in slaughterhouse conditions.

3) The feasibility of monitoring any welfare indicator may vary depending upon the design and layout of the slaughter plant. Therefore, the feasibility of monitoring these indicators can be improved if welfare monitoring is taken into consideration during the design, layout and construction of a new slaughterhouse, or following structural change to existing slaughterhouses.

4) Stakeholders need to be aware that this opinion provides a methodology and a scientifically valid approach to the determination of sample size and sampling protocols. In this regard, the sensitivity, specificity and feasibility of indicators that are relevant to the skill level and facilities of the slaughterhouse should be ascertained and used in estimating an appropriate sample size and developing sampling protocols.

5) The level of competence of the staff influences the feasibility, sensitivity and specificity of the indicators. Therefore, a lack of knowledge and understanding of the physiological basis of the indicators may have contributed to the respondents of the questionnaires rating some indicators as low on sensitivity, specificity and feasibility.

6) Sampling protocols suggested in this opinion are based on sensitivity assessment of indicators by expert survey because there are no (or few) controlled studies under laboratory conditions which have determined the sensitivity of the indicators based on correlation with EEG parameters.

7) In a slaughterhouse, consciousness, unconsciousness and death of the animals are checked throughout the slaughter process by two different categories of operators: (i) the 'personnel', namely the person(s) performing pre-slaughter handling, stunning, shackling, hoisting and/or bleeding, and (ii) the animal welfare officer, the person responsible of the overall animal welfare at slaughter.

CONCLUSIONS ON PENETRATIVE CAPTIVE BOLT STUNNING IN BOVINES

8) To reduce welfare risks due to poor stunning, it is important to detect the animals that are not properly stunned or recover consciousness after stunning. Therefore, it is most important to check periodically indicators with high sensitivity and feasibility in detecting conscious animals.

9) For detecting consciousness in bovines after captive bolt stunning, the sensitivity of the indicators (ability of an indicator to detect conscious animals as conscious) is relevant for animal welfare whereas specificity (ability of an indicator to detect unconscious animals as unconscious) is related more to logistics (personnel have to re-stun the animal).

10) Since unconsciousness should be confirmed from the stunning application until death, this opinion recognises three key stages for monitoring welfare at slaughter: (i) immediately after stunning (between end of stunning and shackling), i(i) during neck cutting (sticking) and (iii) during bleeding.

11) The opinion concludes that a set of indicators (a minimum of two indicators) to be used to detect conscious animals following captive bolt stunning should consist of:

    Key stage 1: posture, breathing, tonic seizure, corneal or palpebral reflex. Additional indicators—muscle tone, eye movements and vocalisation— are also proposed, but their sensitivity is low and they should not be relied upon alone.

    Key stage 2 (during neck cutting): body movements, muscle tone and breathing. In addition, eye movements, corneal or palpebral reflex and spontaneous blinking may also be used.

    Key stage 3 (during bleeding): muscle tone, breathing, spontaneous blinking.

12) In order to develop sampling protocols for monitoring consciousness in bovines after captive bolt stunning, the sensitivity of indicator(s), the threshold failure rate (i.e. tolerance level) for an acceptable proportion of mis-stunning, the size of the slaughter population, the sampling frequency (i.e. sample fraction) and the desired accuracy of the sampling protocol should be determined.

13) In bovine captive bolt stunning, there are two types of risk factors: (i) associated with stun quality and (ii) associated with the quality of the monitoring. Only the latter have an effect on the sampling protocol.

14) Risk factors related to the quality of monitoring may necessitate changes to the sampling protocol applied in the slaughterhouse, from a 'standard' to a 'reinforced' sampling protocol.

CONCLUSIONS ON BOVINE SLAUGHTER WITHOUT STUNNING

15) In the case of slaughter without stunning, it is important to detect the onset of unconsciousness prior to release from restraint, and death prior to dressing, in all animals while assuring identification of conscious and alive animals as such. Therefore it is most important to routinely check indicators that have high feasibility and both high specificity and sensitivity in detecting conscious and alive animals, respectively.

16) During slaughter without stunning, the restraining device, and the presence of blood in the eyes, might affect the feasibility of monitoring the welfare indicators and, as a consequence, the ability to detect unconsciousness.

17) During slaughter without stunning with rotation of animals on to their back for the purpose of neck cutting, the monitoring of some of the welfare indicators is not feasible, as the animal’s head and body are severely restrained and the head is also covered with blood. The experts reported this to be a risk factor leading to reduced feasibility. This concerns eye reflexes, loss of posture and response to nose prick or ear pinch.

18) For monitoring in bovine slaughter without stunning the sensitivity of an indicator (ability to detect conscious animals as conscious) is relevant for animal welfare whereas specificity (ability of an indicator to detect unconscious animals as unconscious) is more related to the logistics (the personnel of the slaughterhouse have to wait longer before releasing the animals from restraint).

19) For monitoring in bovine slaughter without stunning the sensitivity of an indicator (ability to detect alive animals as alive) is relevant for animal welfare whereas specificity (ability of an indicator to detect dead animals as dead) is related to the logistics (the personnel of the slaughterhouse have to wait longer before performing carcass dressing).

20) The opinion concludes that the indicators to be used to detect unconscious animals prior to release from restraint following slaughtering without stunning are breathing and muscle tone. In addition, posture and corneal or palpebral reflex may be used.

21) The opinion concludes that that the indicators to be used to detect dead animals prior to carcass dressing following slaughtering without stunning are bleeding, muscle tone and pupils.

22) In slaughter without stunning, there are two types of risk factors: (i) associated with neck cutting quality and (ii) associated with the quality of the monitoring. However, none of them affect the sampling protocols since all animals have to be checked as required in Regulation (EC) 1099/2009.

23) The formation of aneurysm and occlusion of carotid artery is a major animal welfare concern as it will prolong the time to onset of unconsciousness and death.

RECOMMENDATIONS

GENERAL RECOMMENDATIONS

1) A scientifically based and harmonised set of indicators for use in standard operating procedures in slaughterhouses as well as in monitoring protocols is needed.

2) Further scientific studies should be carried out to ascertain the correlation between the state of consciousness/unconsciousness and death—as measured by brain activity using electroencephalogram—and the behavioural and physiological indicators used to detect unconsciousness and death in order to collect valid information on indicator sensitivity and specificity.

3) The sensitivity of the indicators should be determined under controlled laboratory conditions by correlation with EEG parameters, according to the "Guidance on the assessment criteria for studies evaluating the effectiveness of stunning interventions regarding animal protection at the time of killing" (EFSA AHAW Panel, in press).

4) The level of competence of slaughterhouse staff, which determines the feasibility, sensitivity and specificity of the indicators, should be improved through harmonised education, training and assessment throughout the EU. Until such time as any improvement in sensitivity or specificity resulting from personnel training is objectively demonstrated, the values given in this opinion for calculating the sample size should be considered as a minimum requirement.

5) The procedure for approving the design, layout and construction of a new slaughterhouse or a structural change to an existing slaughterhouse should include as a criterion the feasibility of welfare monitoring throughout the slaughtering process.

6) The animal welfare officer should monitor the effectiveness of the entire stunning and slaughter process, and correct personnel actions or other aspects of the slaughter process if necessary.

7) Since unconsciousness should be confirmed from the stunning application until death, this opinion also suggests checking that the animal is not conscious at each of three key stages: (i) immediately after stunning (between end of stunning and shackling), (ii) during neck cutting (sticking) and (iii) during bleeding.

RECOMMENDATIONS ON BOVINE CAPTIVE BOLT STUNNING

8) During slaughter with stunning, indicators to detect conscious animals should be used to recognise failures (i.e. poor welfare) and apply intervention.

9) A toolbox composed of the following indicators should be implemented to determine consciousness of animals after stunning at all three key stages of the process, to ensure that animals remain unconscious until death occurs.

   Key stage 1: posture, breathing, tonic seizure, corneal or palpebral reflex. Additional indicators—muscle tone, eye movements and vocalisation—are also proposed, but their sensitivity is low and they should not be relied upon alone.

   Key stage 2 (during neck cutting): body movements, muscle tone and breathing. In addition, eye movements, corneal or palpebral reflex and spontaneous blinking may be used.

   Key stage 3 (during bleeding): muscle tone, breathing, spontaneous blinking.

10) In order to develop sampling protocols for monitoring consciousness in bovine captive bolt stunning:

   - The 'personnel' of the slaughterhouse should sample 100 % of the animals immediately after stunning, during neck cutting and during bleeding.

   - The animal welfare officer should periodically sample the slaughter population using the statistical model proposed in this opinion (here referred to as the 'standard' sampling protocol) to calculate the sampling fraction. This fraction is dependent on the test sensitivity, the slaughtered population, the maximum allowed threshold failure rate and the required accuracy.

11) In bovine captive bolt stunning, the ‘standard’ monitoring protocol should be reinforced (here referred to as the ‘reinforced’ sampling protocol) when a conscious animal is detected, or when a risk factor affecting the quality of the monitoring is identified, until the risk is rectified. All animals should be tested during a period represented by one tenth of the slaughtered population.

12) It is recommended that the animal welfare officer should not reduce sampling frequency (sample fraction), as a reduction in sampling fraction ((here referred to as ‘light’ sampling protocol) will immediately reduce the accuracy of the monitoring protocol.

13) Of the recommended indicators above the dashed line in the flow chart, a minimum of two indicators relevant to each key stage should be employed for effective monitoring of the slaughter process.

14) In order to allow effective monitoring, the animals must be able to express behaviours and reflexes associated with consciousness. Consequently, procedures, processes or treatments that could mask the expression of such behaviours (such as electrical immobilisation or electrical stimulation) should not be used prior to confirmation of death in animals.

RECOMMENDATIONS ON BOVINE SLAUGHTER WITHOUT STUNNING

15) The design of the head restraint must be such that the restraint does not obscure the front of the head and it should also allow good access to the eyes to check the corneal (or palpebral) reflex, and to the trachea to assess breathing. In a rotatory restraining system, there should be measures that prevent blood pouring over the animal’s head, impeding effective monitoring.

16) The staff responsible for the assessment of unconsciousness should have the necessary knowledge and skills to differentiate between cessation of bleeding and impeded bleeding by the formation of aneurysm and occlusion of the carotid artery. Animals should be observed closely for the signs of occlusion and appropriate intervention must be applied immediately.

17) Severe restraint and rotation of animals on to their back for the purpose of neck cutting is not conducive to an effective welfare monitoring using key indicators (eye reflexes and response to nose prick or ear pinch) as their heads are covered with blood, and therefore it should be discouraged or phased out.

18) According to Council Regulation (EC) 1099/2009, all ruminants must be mechanically restrained for the purpose of slaughter without stunning, and unconsciousness should be established before releasing the animals from the restraint and death should be confirmed before carcase dressing begins. Therefore, a toolbox of indicators for the determination of unconsciousness and death has been presented in a flow chart, and these indicators should be used during slaughter without stunning. From the toolbox of indicators for use on bovines after slaughter without stunning, it is recommended that breathing, muscle tone, posture, corneal or palpebral reflex should be checked. Their outcomes of unconsciousness should be confirmed before the animal can be released from the restraint. Bleeding, muscle tone and pupils should be checked and their outcomes of death should be confirmed before the animal can be further processed.

19) For slaughter without stunning, 100 % of the animals need to be assessed for unconsciousness and death by checking appropriate indicators i.e. those in toolbox 4 & 5 respectively. The animal welfare officer should confirm unconsciousness and death of the animals as well as the skill and aptitude of the operator in checking the indicators.

REFERENCES

Blackmore DK, 1984. Differences in behaviour between sheep and cattle during slaughter. Research in Veterinary Science, 37, 223–226.

Blackmore and Newhook JC, 1982. Electroencephalographic studies of stunning and slaughter of sheep and calves. 3. The duration of insensibility induced by electrical stunning in sheep and calves. Meat Science, 7, 19–28.

Cannon RM, 2001. Sense and Sensitivity – designing surveys based on an imperfect test.. Preventive Veterinary Medicine, 49, 141-163.

Cook CJ, Maasland SA, Devine CE, Gilbert KV and Blackmore DK, 1996. Changes in the release of amino acid neurotransmitters in the brains of calves and sheep after head-only electrical stunning and throat cutting. Research in Veterinary Science, 60, 255–261.

Daly CC, Gregory NG and Wotton SB, 1987. Captive bolt stunning of cattle: effects on brain function and role of bolt velocity. British Veterinary Journal, 143, 574–580.

Daly CC, Kallweit E and Ellendof F, 1988. Cortical function in cattle during slaughter: conventional and captive bolt stunning followed by exanguination compared to Shechita. Veterinary Record, 122, 325–329.

EFSA (European Food Safety Authority), 2004. The welfare aspects of the main systems of stunning and killing the main commercial species of animals. The EFSA Journal 2004, 45, 1-29.

EFSA (European Food Safety Authority), 2006. The welfare aspects of the main systems of stunning and killing applied to commercially farmed deer, goats, rabbits, ostriches, ducks, geese. The EFSA Journal 2006, 326, 1-18.

EFSA AHAW Panel (EFSA Panel on Animal Health and Welfare), 2013. Guidance on the assessment criteria for studies evaluating the effectiveness of stunning interventions regarding animal protection at the time of killing. EFSA Journal 2013;11(12):3486. doi: 10.2903/j.efsa.2013.3486

EFSA AHAW Panel (EFSA Panel on Animal Health and Welfare), in press. Report form public consultation concerning the scientific opinion on monitoring procedures at slaughterhouses.

Gardner IA, Stryhn H, Lind P and Collins MT, 2000. Conditional dependence between tests affects the diagnosis and surveillance of animal diseases. Preventive Veterinary Medicine, 45, 107–122.

Grandin T, 1994. Euthanasia and slaughter of livestock. Journal of the American Veterinary Medicine Association, 204, 1354–1360.

Gregory NG, 1998. Physiology of stress, distress, stunning and slaughter. In: Animal welfare and meat science, 64–92. CABI: Wallingford, Oxon, UK.

Gregory NG, Fielding HR, von Wenzlawowicz M and von Holleben K, 2010. Time to collapse following slaughter without stunning in cattle. Meat Science, 85, 66–69.

Lambooij E and Spanjaard W, 1981. Effect of the shooting position on the stunning of calves by captive bolt. Veterinary Record, 109, 359–361.

Lambooij E, van der Werf JTN, Reimert HGM and Hindle VA, 2012. Restraining and neck cutting or stunning and neck cutting of veal calves. Meat Science, 91, 22–28.

Limon G, Guitian J and Gregory NG, 2010. An evaluation of the humaneness of puntilla in cattle, Meat Science, 84, 352–355.

Michiels C, 2004. Physiological and pathological responses to hypoxia. American Journal of Pathology, 164, 1875–1882.

Muir WW, 2007. Considerations for general anesthesia. In Lumb & Jones' veterinary anesthesia and analgesia, 4th edn. Eds Tranquilli WJ, Thurmon JC, and Grim KA. Blackwell Publishing, Oxford, UK, 7–30.

Newhook JC and Blackmore DK, 1982. Electroencephalographic studies of stunning and slaughter of sheep and calves-part 2: The onset of permanent insensibility in calves during slaughter. Meat Science, 6, 295–300.

O'Connor A, Dzikamunhenga R, Sargeant J, Glanville J and Wood H, in press. Preparatory work for future development of four scientific opinions on monitoring procedures at slaughterhouses. EFSA supporting publication, in press.

Pallis C, 1982a. ABC of brain stem death. Diagnosis of brain stem death I. British Medical Journal, 285, 1558–1560.

Pallis C, 1982b. ABC of brain stem death. Diagnosis of brain stem death II. British Medical Journal, 285, 1641–1644.

Pallis C, 1982c. ABC of brain stem death. From brain death to brain stem death. British Medical Journal, 285, 1487–1490.

Pallis C, 1982d. ABC of brain stem death. Reappraising death. British Medical Journal, 285, 1409–1412.

Rosen SD, 2004. Physiological insights into Shechita. Veterinary Record, 154, 759–765.

Sellke P, in press. Expert Knowledge Elicitation on Indicators for Success of Stunning and Killing. EFSA supporting publication, in press.

Terlouw EMC, Bourguet C and Deiss V, 2012. Origines des mouvements présentés par les bovins après l'étourdissement et pendant la saignée. Proceedings 3R, Paris, 5–6 December 2012.

won Holleben M, von Wenzlawowicz N, Gregory H, Anil A, Velarde P, Rodriguez B, Cenci Goga B, Catanese B and Lambooij E, 2010. Report on good and adverse practices—Animal welfare concerns in relation to slaughter practices from the viewpoint of veterinary sciences. Available online: http://www.dialrel.eu/images/veterinary-concerns.pdf.

Zeman A, 2001. Consciousness. Brain, 124, 1263–1289.

GLOSSARY

GLOSSARY FOCUSED ON KEYWORDS

Outcome of a welfare indicator. This is the result of check performed using an indicator based on which the animal is considered conscious or unconscious, or alive or dead.

Welfare indicator. This is an observation used to obtain information on an animal's state of consciousness/unconsciousness or life/death. In this opinion, all the indicators are animal-based observations.

GLOSSARY FOCUSED ON THE MODEL

Accuracy of the sampling protocol. This is the percentage of situations in which the sampling protocol was applied and served its purpose, i.e. raising an alarm if there were more ineffectively stunned animals than the prescribed threshold failure rate would allow. This corresponds to confidence level in freedom from disease methodologies.

Sampling fraction. This is the proportion of the slaughter population which is assessed in the sampling protocol.

Sensitivity of the indicators. As defined previously, this is the percentage of truly conscious animals detected as conscious by the indicator. This corresponds to diagnostic test sensitivity in freedom from disease methodologies.

Slaughter population. A group of animals slaughtered under the same circumstances as determined by risk factors (see Table 10).

Threshold failure rate for proportion of mis-stunned animals. This specifies the minimum proportion of animals that are ineffectively stunned, but which will still be detected by the sampling protocol. This corresponds to design prevalence in freedom from disease methodologies.

GLOSSARY FOCUSED ON INDICATORS

DEFINITIONS OF THE INDICATORS FOR CAPTIVE BOLT STUNNING IN BOVINES

Breathing. Effective stunning will result in the immediate onset of apnoea (absence of breathing). Ineffectively stunned animals and those recovering consciousness will start to breathe in a pattern commonly referred to as rhythmic breathing, which involves a respiratory cycle of inspiration and expiration Rhythmic breathing can be recognised from regular movement of the flank and/or mouth and nostrils. Recovery of breathing, if not visible through these movements, can be checked by holding a small mirror in front of the nostrils or mouth and watching for the appearance of condensation on the mirror as a result of the expiration of moist air.

Corneal reflex. The corneal reflex is elicited by touching or tapping the cornea. Ineffectively stunned animals and those recovering consciousness will blink in response to the stimulus. Unconscious animals may also intermittently show a positive corneal reflex.

Eye movements. Eye movements and the position of the eyeball can be recognised from close examination of eyes after captive bolt stunning. Correctly stunned animals will show fixed eyes as a result of concussion of the brain, and this can be recognised from wide-open and glassy eyes with clearly visible iris/cornea in the middle. Ineffectively stunned animals and those recovering consciousness will show nystagmus (spontaneous rapid side-to-side movements of the eyeballs) or rotation of the eyeball. Rotation of eyeball can be recognised from the appearance of mostly sclera, with little or no iris/cornea being visible.

Muscle tone. Unconscious animals will show general loss of muscle tone, and this can be recognised from the completely relaxed legs, floppy ears and tail, and relaxed jaws with protruding tongue.

Palpebral reflex. The palpebral reflex is elicited by touching or tapping a finger on the inner/outer eye canthus or eyelashes. Ineffectively stunned animals and those recovering consciousness will blink in response to the stimulus.

Posture. Effective stunning will result in brain concussion, which can be recognised from the immediate loss of posture leading to collapse of the animal to the floor. Ineffectively stunned animals, on the other hand, will fail to collapse or will attempt to regain posture after collapse. Some ineffectively stunned animals (as may occur, for example, if shooting position is wrong) may lose posture as a result of the impact of the bolt and remain collapsed in the restraining/stunning box.

Pupillary reflex. The pupillary reflex can be elicited by focusing/shining a torch light at the pupils. Live and conscious animals will show pupillary constriction (miosis) in response to light. Deeply unconscious animals will not show a positive reflex.

Spontaneous blinking. Animal opens/closes eyelid on its own (fast or slow) without stimulation. Ineffectively stunned animals and those recovering consciousness will blink spontaneously.

Responses to a nose prick or ear pinch. The absence of response to a painful stimulus, such as a pin prick to the muzzle (area between external nostrils) or the ear with a sharp instrument, indicates unconsciousness following stunning.

Tonic–clonic seizures. Effective stunning will result in tonic–clonic seizures, which can be recognised by an arched back and rigidly flexed legs under the body, followed by clonic seizure manifesting as leg kicking or paddling. Tonic seizure may end, and therefore not seen, if there was a long delay between the end of captive stunning and removal of the animal from restraining/stunning box.

Vocalisation. Conscious animals may vocalise, and therefore purposeful vocalisation, such as grunting, bellowing or mooing, can be used to recognise ineffective stunning or recovery of consciousness. However, not all conscious animals may vocalise.

DEFINITIONS OF THE INDICATORS PRIOR TO RELEASE FROM RESTRAINT FOR SLAUGHTER WITHOUT STUNNING IN BOVINES

Breathing. Loss of consciousness following slaughter without stunning will eventually lead to cessation of rhythmic breathing. Rhythmic breathing can be recognised from the regular flank movement. Therefore, the sustained absence of rhythmic breathing (absence of respiratory cycle – inspiratory and expiratory movements) can be used as an indicator of the onset of unconsciousness. The trachea is severed during slaughter without stunning, and therefore signs of rhythmic breathing cannot be observed at the nostrils or mouth. Cessation of breathing can be indicated by the absence of air bubbles in the liquid blood or sera accumulating at the neck wound or by the absence of any flank movements suggestive of breathing.

Corneal reflex. The corneal reflex is elicited by touching or tapping the cornea. Permanent loss of the corneal reflex can be used as an indicator of unconsciousness.

Fixed eyes. Permanent loss of consciousness following slaughter without stunning will lead to fixed eyes, and this can be recognised from wide-open and glassy eyes with clearly visible iris/cornea in the middle.

Muscle tone. Unconscious animals will show a general loss of muscle tone, and this can be recognised from the completely relaxed legs, floppy ears and tail, and relaxed jaws with protruding tongue.

No full eyeball rotation. Unconscious animals have fixed eyes and therefore do not show rotation of the eyeballs.

Nystagmus. Unconscious animals have fixed eyes and therefore do not show nystagmus, i.e. no spontaneous, rapid, side-to-side (twitching) movements of the eyeballs.

Palpebral reflex. The palpebral reflex is elicited by touching or tapping a finger on the inner/outer eye canthus or eyelashes. Permanent loss of the palpebral reflex can be used as an indicator of unconsciousness.

Posture. Permanent loss of posture can be used as the earliest physical sign of the onset of unconsciousness following slaughter without stunning of an unrestrained/free-standing animal. An animal that has lost consciousness irreversibly will make no attempt to regain posture. Animals may lose posture as a result of loss of consciousness but subsequently recover consciousness because of the formation of carotid aneurysm/occlusion and sustained blood supply to the brain via the vertebral artery. These animals may or may not attempt to regain posture. Loss of posture cannot be determined in animals that are severely restrained and/or rotated. Conscious animals may lose posture if the floor is slippery and may or may not attempt to regain posture.

Pupil size. Dilated pupils (midriasis) are indication of death.

Pupillary reflex. The pupillary reflex can be elicited by focusing/shining a torch light at the pupils. Live and conscious animals will show pupillary constriction (miosis) in response to light. Unconscious animals will not show a positive pupillary reflex.

Responses to a nose prick or ear pinch. The absence of response to a painful stimulus, such as a pin prick to the muzzle (area between external nostrils) or the ear with a sharp instrument, indicates unconsciousness following stunning.

Spontaneous blinking. Unconscious animals do not blink.

Vocalisation. Conscious animals may vocalise, and therefore purposeful vocalisation, such as grunting, bellowing or mooing, can be used to recognise ineffective stunning or recovery of consciousness. During slaughter without stunning, the trachea is also severed, and therefore animals cannot vocalise; however, blood enters trachea and movement of air through the blood or serum generates bubbling and gurgling noises that may be falsely taken as vocalisation.

DEFINITIONS OF THE INDICATORS PRIOR TO DRESSING FOR SLAUGHTER WITHOUT STUNNING IN BOVINES

Cardiac activity. Onset of death leads to permanent absence of cardiac activity (absence of heart beat), which can be ascertained using a stethoscope where possible.

End of bleeding. Slaughter eventually leads cessation of bleeding, with only minor dripping, from the neck cut wound, and therefore the end of bleeding can be used as an indicator of death. However, formation of aneurysm and occlusion of the carotid artery may prevent blood flow from the neck cut wound, and this should not be mistaken for the end of bleeding. Aneurysm and occlusion of the carotid artery may be visible and recognised from blood squirting out.

Pulse rate. Onset of death leads to permanent loss of pulse, which can be ascertained physically by pressing the arteries in an extremity where possible.

Pupil size. Dilated pupils (midriasis) are an indication of death.

Relaxed carcass. Onset of death leads to a complete and irreversible loss of muscle tone, which can be recognised from the limp carcasses.