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Appendixes

The appendixes provide more detailed information on various topics relating to village chicken production.

Appendix 1

How to use this manual as part of a training course for livestock officers, extension agents, community animal health workers or farmers

The information contained in this manual can be used to assist participatory training and extension activities with livestock officers, extension agents, community animal health workers and poultry farmers. Participatory technology development and farmer field-school approaches are particularly suitable for the application and trial of information and techniques described here.

As noted in various sections of this manual, the constraints to local poultry production may include predation, inadequate nutrition, inefficient flock management and various diseases. The appendixes contain information that will assist with cost-efficient disease surveillance and the interpretation of serological results.

The participatory tools presented in Appendix 2 can be used to:

- identify and prioritise local constraints to village poultry production (community animal health worker and farmer-oriented training)
- identify and prioritise local constraints to disease surveillance, prevention and control (livestock officer and extension agent-oriented)
- identify available and affordable local resources to deal with the constraints
- plan trials to assess the usefulness of different prevention and control options under local conditions
- design participatory monitoring and evaluation activities to enable the definition of indicators and methods of verification by participants, implementers and donors.

Appendix 2

Participatory community exercises for identifying problems associated with village chicken production

Exercises

1. Awareness-raising activities and descriptions of the community and chicken production
   - (a) Wellbeing stratification exercise
   - (b) Community structures (Venn diagrams)
   - (c) Family roles related to chicken raising
   - (d) Ranking of activities according to their contribution to household income
   - (e) Chicken-raising calendar
2. Problem identification
   - (a) Ranking of problems related to chicken raising
   - (b) Focus group discussions
3. Dreams realised or visioning

Exercises 1(a) and 1(b) will help to identify the characteristics of social groups within a community and how various community structures interact. Exercises 1(c), (d) and (e) are related to identifying the importance of chicken production and the roles and activities associated with raising chickens. These last three exercises could also be used again later, to help evaluate and identify the changes that have occurred as a result of new chicken-management practices.

Exercises 2(a) and (b) can be used to identify problems associated with chicken production. They can also be used later, to help evaluate changes as a result of new chicken-management practices.

The third exercise may help the community to identify and envision potential benefits of improved village chicken production.

1(a) Wellbeing stratification exercise

Time required

Approximately 1 hour

Purpose

To identify three different socioeconomic groups in the target population, representative of the well off, the less well off and the poor. This information can

be used to identify households from each of the social groups. People from these different groups could be interviewed to determine the role that chickens play in their production system and to identify constraints to production within each of the groups. This process will help to ensure that extension activities are meeting the needs of a wide range of people within the community.

Materials

Flip chart and felt pen

Process

Organise a meeting of around 30–60 people of the village representative of the well off, the less well off and the poor, and men and women.

1. The facilitator introduces the team, the objective and the exercise.
2. The facilitator asks participants to describe the characteristics of three social groups in the community: well off, less well off and poor. The information is listed on the flip chart.
3. The discussion should flow from one issue to the other logically. The following guidelines list the types of questions that can be asked to guide the discussion.

   Availability of food

   Do all households eat the same number of meals? Are there times of the year when some families do not have enough food? How often do families eat meat?

   Agricultural and livestock practices and assets

   Do all households own land? Do all households cultivate their own land? Do some households rent land? Do all households own hand tools? What types of households own oxen, ox ploughs and ox carts? What types of households own tractors? How do different households cultivate land (hand tools, ox ploughing, tractor or hired labour)? What types of food crops do different households grow? What types of livestock do different households own? Do all households own livestock? Do all households use agricultural inputs such as veterinary medicines and chemicals (vaccines, antibiotics, fertilisers, insecticides, herbicides, fungicides etc.)?

   Household income

   What are the major sources of income? What types of crops are grown? What types of livestock are raised? How important are chickens as a source of income? What are other sources of on-farm income (e.g. beer brewing)? Are there sources of off-farm income (e.g. school teacher, working on other farms)? How important is on-farm and off-farm income in different households?

   Household assets

   What different types of houses are there? How many households have thatched roofs or tin roofs? How many households live in mud and wattle

houses, or houses made of mud bricks or concrete blocks? Do all houses have a latrine? Do all households use mosquito nets? What are the main items of furniture owned in different households? What are the main kitchen utensils used in each house?

Social characteristics

What different types of households are there? Are there, for example, households that are primarily female or without adult male members; households with only old people; households with sick and physically infirm people; households with only children; households with young families; extended polygamous households; extended monogamous households? How many households pay local fees and levies?

4. The facilitator should either interpret the ideas coming from the participants to describe the characteristics of each social group or, alternatively, the characteristics should first be listed randomly and the second step would be to organise these different characteristics into the categories of well off, less well off and poor in a second stage of discussion.
5. If the facilitator wishes to work with families in each of these categories it is a good idea not to name the groups as 'rich or well off' or 'poor' etc., as this can be embarrassing or demeaning for participants. It is better to identify a main characteristic of each group, such as the number of cattle owned or the area of land farmed and categorise families accordingly.

1(b) Describing community structures (Venn diagrams)

Time required

Approximately 1 hour

Purpose

To help the community and the facilitators understand the organisational structures that exist within the community and how they relate to each other. This may help to determine the best ways to work with the community and to avoid conflicts between existing groups.

Materials

Flip chart and pen; scissors; tape or glue

This activity can be carried out with village leaders and also with same-sex meetings of 20 men and 20 women of different social groups. The meetings can be held on different days.

Process

- Using a sheet of paper from a flip chart, ask the participants to list all of the groups and structures (formal and informal; official and unofficial) within their village.

- Using a new sheet of paper, draw a circle for each group/structure in the list and write the name of that group/structure in the circle. The more important the group, the larger should be the circle.
- Cut out each circle.
- Arrange the circles on a fresh sheet of paper to illustrate the overall system and to show relationships between the different parts. Circles can overlap, be far apart, close together, or within other circles etc.
- Display and explain the system developed to the other groups so that differences can be discussed. Analyse key differences between the groups and the underlying causes.

Notes

This can be a very illuminating exercise for the community, as certain aspects of their village life and organisation may be explicitly revealed for the first time. It will also show the different perceptions of different groups. It may help to highlight different roles, responsibilities and linkages, pointing to areas of conflict and dispute as well as to ways of resolving them.

Different groups may produce completely different diagrams. These can then be discussed to help resolve conflicts and encourage linkages. However, it may not be possible to pull all of these systems together into something that represents them all. Diversity needs to be accepted.

1(c) Identifying family roles relating to chicken raising

Time required

Approximately 1 hour

Purpose

- To understand the roles of different family members so that meetings and training can be conducted with the appropriate family member(s).

Materials

Flip chart and felt pen

Process

- Draw the following table (Table A1.1) on a flip chart and leave space at the bottom for adding extra tasks.
- Either in one large group or in groups divided into men and women, discuss the tasks listed in the table and ask participants to add or remove tasks according to what is normally done to look after village chickens.
- Ask participants to identify who performs the different activities related to chicken raising and to fill in the table.
- Discuss the main findings and assess who should be most involved in training or activities related to chicken raising to ensure that the person involved with the activity is adequately informed and is in charge of it.

Table A2.1 Family activities related to chicken raising

Activities	Adult men	Adult women	Boys	Girls	Older men	Older women
Large species of livestock						
Small species of livestock						
Chicken raising						
Who gives feed?						
Who gives water?						
Who built the chicken house?						
Who prepared the place for the hen to hatch?						
Who cleans the house?						
Who receives information on chicken raising?						
Who owns the birds?						
Who decides when to sell birds?						
Who decides when to sell eggs?						
Who decides whether to vaccinate?						
Who decides on need for and level of supplementary feed?						
Who opens and closes the chicken house door?						
Who collects the eggs?						
Who slaughters birds and prepares them for consumption?						
Who can eat birds?						
Who can eat eggs?						
Who decides when to eat birds or eggs?						
Who takes care of sick birds?						
Who decides if vaccination was successful?						

1(d) Ranking of activities according to their contribution to household income

Time required

Approximately 30 minutes

Purpose

- To identify how important chicken raising is in different households in comparison to other enterprises
- To understand the reasons for the relative importance of chicken raising
- To measure the change in importance attached to chicken raising as improved management techniques are introduced

Materials

Flip chart and pens; A4-size paper; drawings; stones or beans

Process

- Organise two meetings, one with 20 men, the other with 20 women, the participants in each case being drawn from a range of social groups. The meetings can be held on the same or different days.
- Each group should be divided into the three social categories (remembering not to ask people to divide according to categories such as 'rich' or 'poor', but rather according to less sensitive descriptions such as those who own many cattle, a few cattle or no cattle.
- Conduct one of the following two exercises with each group:

1. Simple ranking

(a) The facilitator introduces the team and explains that the objective is to identify the importance of chicken raising relative to other household activities. The facilitator explains that everybody should contribute with their ideas. (Register the total number of participants in the group.)

(b) Identify the major crops, animal enterprises and other activities in the participants' livelihood strategies.

(c) Either use existing drawings or draw the animals, crops and activities on cards.

(d) Distribute the same number of stones or beans to each of the participants. Ask each of the participants to put a number of stones on the drawing according to their importance in their household livelihood. Ask each participant to justify their ranking. (Verify that each participant uses all the stones distributed.)

(e) At the end of the exercise, count the stones on each of the activities. Calculate the contribution of each activity as a percentage of the total. (Divide the number of stones in each drawing by the total number of stones distributed and multiply by 100.)

(f) Discuss with the participants the result obtained. Verify that it corresponds to their perception of the reality. Discuss its implications for the project.

2. Preference ranking

Preference ranking is a variation of simple ranking and can be used as an alternative to it. It involves the participants assessing the relative importance of different farm enterprises using criteria they themselves identify. The ranking can be undertaken using a matrix with farm enterprises on the horizontal axis and the selected criteria on the vertical axis. An example is given in Table A2.2.

The advantage of preference ranking over simple ranking is that the participants select the criteria for ranking and so it is easier to understand the reasons for the ranking. The disadvantage is that it is more difficult to facilitate.

The following steps are involved in farm enterprise preference ranking:

(a) The facilitator introduces the team and explains that the objective is to identify the importance of chicken raising relative to other household activities. The facilitator explains that everybody should contribute their ideas. (Register the total number of participants in the group.)

(b) Ask participants to identify the six most common farm enterprises. Ensure that chicken raising is included in the list.

(c) Ask participants to select criteria for ranking the importance of each enterprise by asking the following questions: 'What is good about this enterprise?' 'What else?' (Continue until there are no more replies.) Then ask: 'What is bad about this enterprise?' 'What else?' (Continue until there are no more replies.)

(d) List all the criteria.

(e) Turn negative attributes into positive ones (e.g. 'prone to disease' becomes 'disease resistant'; 'a lot of work' becomes 'little work')

(f) Help participants to draw up a matrix with criteria listed down the side of the matrix and enterprises along the top. (Where possible, use symbols for the row and column titles—either prepared before or prepared at the meeting.)

(g) Using a five-point scoring system in which five points is the highest and best score, in a group exercise rank each of the enterprises for each of the criteria.

(h) When the exercise is completed, cross-check the ranking of each enterprise, by asking a question to confirm its ranking (e.g. 'It seems that chicken enterprises are/are not very important for household income. Is this correct?').

(i) Follow up the ranking results by a discussion to explore different viewpoints.

(j) This exercise will require two facilitators; one to assist the participants in the exercise and the other to record the discussions.

Table A2.2 gives an example of how a preference ranking matrix for the above exercise might look. (The values shown are examples only.)

Table A2.2 An example of a preference ranking matrix

	Chickens	Goats	Cashew	Maize	Beans	Cassava
Profitable	1	2	4	3	4	3
Disease free	1	1	1	3	3	4
Easy to sell	4	4	4	3	4	2
Low labour needs	5	4	3	2	2	3
Available when food for the family is in short supply	4	5	1	1	1	4
Total	15	16	13	12	14	16

This table indicates that cashew and maize may be less important than the other four activities.

1(e) Chicken-raising calendar

Time required

Approximately 2 hours

Purpose

To identify when the main activities relating to chicken raising occur throughout a year. To provide a structure to allow an exploration of important issues relating to chicken husbandry practices; chicken and egg consumption and marketing; knowledge of, attitudes to and responses to Newcastle disease (and/or other important poultry production constraints); and community ideas for improving any existing ND control activities. The data collected will complement the data collected in the baseline survey.

Frequency

To be undertaken in the initial participatory rural appraisal (PRA) and annually thereafter.

Materials

Flip chart and pens; drawings of different activities (the participants can also draw these); glue; notebook

Process

Two facilitators are required, one to lead the discussion, the other to take notes.

Organise meetings of same-sex residents, with around 20 people in each group. The meetings can be held one after the other or on different days.

1. A facilitator introduces the team and explains the objective of the exercise: to discuss chicken-raising activities.
2. When an activity occurs at a specific time of the year, it is put on the calendar. Other information is collected in a notebook.
3. Residents draw an annual calendar divided into 12 monthly columns on the flip chart. The facilitator should assist if required.
4. The discussion should flow logically from one issue to the other. Examples follow of the types of questions that can be asked to guide the discussion:

Chicken ownership

- Does everybody own or look after chickens? Who does not have any and why? How many chickens does each person own or look after? What is the normal (average) number of chickens owned or looked after by a household?
- In which months do people have more chickens? Why? Is it during harvest period? When do they have less? Is it at the end of the hungry period? (Mark on the calendar (see Table A1.3) the month when households have most chickens and when they have the least chickens.)
- Who owns the chickens in the household? Who takes care of them?

Chicken husbandry practices

- What do chickens eat?
- Where do chickens sleep at night? How secure is the chicken house?
- What are the main problems with chicken raising? Diseases, names of the diseases, explanation of the origin and transmission of the diseases, other problems? (Record the information about diseases in the notebook and mark the appropriate months on the calendar.)

Newcastle disease

- When do ND outbreaks usually occur? When are the peaks of the disease? (Record the information about ND in the notebook and mark the appropriate month(s) on the calendar.)
- How do you recognise ND (what are its characteristics)? What prevention and treatment methods do you use? Who gives the treatment? (Record the information about when treatment is normally given in the notebook and mark the appropriate month(s) on the calendar.)
- Repeat for other significant diseases in the area.

Agricultural calendar

- Which months are the rainy seasons? In which months are the main agricultural activities undertaken? (Record the information in the notebook and mark on the calendar the months in which the various activities normally occur.)

Consumption of chicken products

- When do people usually eat more chickens and eggs? Why? Is it during feasts or before a ND outbreak? (Record the information in the notebook and mark the relevant months on the calendar.)
- Who usually eats the different parts of the chicken? Why? Who eats most of the chicken? At what age can a child start eating eggs or chicken? Why? Do pregnant women eat eggs and chicken? Why or why not? (Record the information in the notebook.)

Chicken and egg sales

- What is the price of a chicken and of an egg in the different periods of the year? Are they (birds and eggs) sold by piece or by weight? When is the price lower and when is it higher? Why? (Record the information in the notebook and mark the relevant months on the calendar.)
- Where do people usually sell chickens and eggs? Why? Is the price the same in the different locations? (If there is a seasonal variation, record the information in the notebook and mark the relevant months on the calendar.)
- Who sells eggs and chickens (men, women, children)? What do they do with the money? Who controls the money from the sale of chickens? (Record the information in the notebook.)
- Who makes the decision to eat or sell? (If there is a seasonal variation, record the information in the notebook and mark the relevant months on the calendar.)

Newcastle disease vaccination

- Have you heard about ND vaccination? With what type of vaccine: La Sota, I-2, some other type? What is it? Who stores or looks after the vaccine? Who organises the vaccination? Is a campaign or ongoing vaccination approach used? Is ND vaccine available in the village? (If there are campaigns against ND, record the information in the notebook and mark the relevant months on the calendar.)
- How effective was the vaccination? Did all the chickens that were vaccinated survive the ND outbreak? Was there any difference in deaths from ND between vaccinated and non-vaccinated birds? How do you think the vaccination process can be improved? (If the timing of existing vaccinations should be altered, record the information in the notebook and mark the recommended time on the calendar.)

Table A2.3 **An example of a chicken-raising calendar summarising data gathered in 2003 before vaccination with Newcastle disease vaccine began in Mtwara region, Tanzania**

Issues	J	F	M	A	M	J	J	A	S	O	N	D
More chicken die of lice						x	x	x				
ND outbreak									x	x	x	x
Rain	x	x	x	x							x	x
Weeding		x	x									
Land preparation										x	x	
Harvest					x	x	x	x	x			
Few chickens	x	x	x					x	x	x	x	x
Hunger period	x	x	x	x					x	x	x	x
Many chickens						x	x	x				
More chickens are eaten								x	x	x	x	x
High price for chickens	x	x	x	x	x							
Low price for chickens							x	x	x	x	x	x

2(a) Ranking of problems related to chicken-raising

Time required

Approximately 30 minutes

Purpose

To rank the relative importance of the different problems facing chicken raisers over the life of the project.

Frequency

To be undertaken in these participatory community exercises and annually thereafter.

Materials

Flip chart and pen; drawings; stones or beans

Process

This activity can be carried out with separate meetings of up to 20 men and 20 women, participants in each case being drawn from the three different social groups. The meetings can be held on different days. The activity should be conducted annually.

1. The facilitator presents the team and explains that the objective of the exercise is to discuss the problems related to chicken raising and to rank them according to their importance. Everybody is asked to contribute their ideas. (Write down the total number of participants.)

2. Discuss what are the main problems with chicken raising.
3. Use existing drawings or ask the participants to draw the different activities.
4. Distribute the same number of stones or beans to each of the participants. Ask each of the participants to put a number of stones on the drawing according to their importance. Ask each participant to justify their ranking. (Verify that each participant uses all the stones distributed.)
5. At the end of the exercises, count the stones on each of the cards/ drawings. Calculate the percentage of stones placed on each card/ drawing by dividing the number of stones in each drawing with the total number of stones distributed and multiplying by 100 (Table A2.4). Convert the percentages to a ranking, with 1 being the highest ranking (highest percentage) and record the rankings on the flip chart (Table A2.4).
6. Discuss with the participants the results obtained. Verify if it corresponds to their perception of reality. Discuss its implications for the project.

Note: This exercise could also be undertaken using pair-wise ranking where each problem is compared in importance to every other problem.

Table A2.4 An example of problem ranking by men and women in 2003 and 2005 in Dodoma region, Tanzania. Vaccination against Newcastle disease began in 2003.

Issue	2003	2003	2005	2005
	Men	Women	Non vaccinating	Vaccinating
Newcastle disease	1	1	1	
Predation/theft	2	9		
Coughing	6	5		
Housing	5	2		4
External parasites	3	3		2
Fowl pox	8	4		
Diarrhoea	7	7		
Feed	8	7		
Marketing	3	–		
Swollen liver		6	3	
Swollen eyes/head			2	1
Death of chicks				3
Worms				4
Infectious coryza			4	

2(b) Focus group discussions

Time required

Approximately 1–2 hours

Introduction

Focus group discussions (FGDs) are one of the main tools to be used to collect data about the implementation and effectiveness of vaccination campaigns.

Focus group discussions are facilitated discussions held with small groups that have either homogeneous or heterogeneous views. Group size should be kept to between 8 and 12 persons. The discussions usually last 1–2 hours and have many potential uses, including:

- to serve as a forum for addressing a particular issue to highlight various concerns, conflicting interests and common ground among different groups
- to provide an opportunity to cross-check information collected using other techniques
- to obtain a variety of reactions to hypothetical, planned or actual interventions.

The skill of the facilitator is an important element in the success or failure of FGDs. The person who guides the focus group uses group-process skills to ensure that all the participants can speak openly and to direct the discussion to the relevant topic.

The most useful outputs of these discussions are in the form of qualitative insights and direct quotations illustrating the views of the group's members.

Focus group discussions will be used on the project to empower communities and to collect information for strategic planning and impact assessment.

The next sections outline the overall approach and tips for facilitators on how to prepare for and conduct FGDs.

Focus group discussions can also be used to collect information about gender, poverty alleviation, the extent of community participation and many other areas.

Purpose

Focus group discussions provide forums at which community members can discuss the performance, effectiveness and impact of vaccination campaigns, and make recommendations for improvements. The results of the discussions should be communicated to vaccinators, supervisors and national coordinators and other service-delivery agencies so that planning, implementation, monitoring and effectiveness can be improved.

Frequency and timing

Focus group discussions should be held at the end of every vaccination campaign in all pilot villages where vaccination campaigns are conducted.

Participants

Focus groups comprising between 8 and 12 people should be formed. A person trained by the project will facilitate each group. Two groups will be formed in every project village, one comprising men the other women. The groups should be representative of the three wealth categories in each village, and of vaccinating and non-vaccinating farmers.

Topics for discussion

The main topics of discussion will be: How effective was the last vaccination campaign? How can implementation be improved? What benefits have been created as a result of the vaccination campaigns? Who are the beneficiaries? The project will provide facilitators with general checklists of topics to be covered for each campaign; see example in the box below.

First Newcastle disease vaccination campaign, checklist of subtopics

- Timing of the vaccination campaign
- Methods used in the campaign
- How can campaigns be improved?
- Numbers of chickens vaccinated
- Numbers of farmers vaccinating chickens
- Reasons why some farmers vaccinated and others did not vaccinate
- Knowledge about vaccinations among group members
- Attitude to vaccinations among group members

Length of group discussions

Discussions should last 1–2 hours.

Recording of the discussions

The facilitator should keep a record of the discussion and they should leave a copy with the community. If possible, somebody should assist the facilitator to record the discussions.

Reporting of focus group discussions

The facilitator should report on the FGDs to district-level personnel who should, in turn, report to regional authorities.

Tips on how to prepare and conduct focus group discussions:

- Have a clear purpose for the group discussion, based on the general project checklist.

- Prepare a checklist of issues to be covered in the discussion, based on the general checklist prepared by the project. Prepare prompts that can be used to open up areas that need to be discussed.
- With the help of local leaders and key informants (including the vaccinator and supervisor), identify participants. Ensure all required subgroups are represented.
- Advise people well in advance when the discussions will be held.
- Ensure there is a comfortable and pleasant atmosphere. Arrange snacks and drinks.
- Start the discussion with enough authority to keep the discussion on track, but with sufficient sensitivity to include everybody in the discussions. It is a good tactic to say that you are not an expert on the issues that are being discussed and that you want the participants to help you understand the issues better.
- Try to identify which issues are of general concern to the group and which issues are more controversial or personal in nature.
- When important issues have been agreed by the group, ensure that you fully understand them. A good way to make sure you understand is to paraphrase what they have agreed and ask them if you have understood properly.
- Look for potential spokespersons from different focus groups who could be asked to meet together to summarise the concerns of their groups and discuss differences between the groups.
- The facilitator would identify prompts and questions for each of the subtopics to assist spokespersons to facilitate the discussion.

3 Dreams realised or visioning

Time required

Approximately 1 hour

Purpose

To identify how participating stakeholders expect the project will benefit them, and what other changes they expect from the project over a longer time scale; e.g. 10 years. To identify indicators and to discuss how to measure the benefits and changes expected.

Process

The following method could be used.

- In one large group, start by asking participants how they would like things to be as a result of improved chicken production in the future. Ask the participants to reflect individually on the following question: How would you describe *the ideal situation we wish to achieve here in 10 year's time as a result of improved chicken production*? (10 minutes)

- Divide into subgroups (by gender if appropriate) and write down on cards or create symbols for the visions. (15 minutes)
- Meet in the large group to discuss the visions, to identify how best the community can assess the progress being made in achieving the visions and to agree on how the community will keep track of the changes. (30 minutes)
- The development of the dreams/indicators needs to be properly recorded so that a time series of information is stored. It is important to compare the current dreams with those identified previously, to discuss why changes have occurred and to what extent changes have been caused by project activities or external factors.

Appendix 3

Collection of blood samples

The examination of serum can reveal previous contact of the sampled bird with a particular infectious agent (ND virus, for example) and provide information about the success of vaccination. It takes some time (generally about 2 weeks) after vaccination or contact with an infectious agent before antibodies to the agent are found in the blood.

Further information on serology is given in Section 5 of Appendix 5.

Blood samples can be collected from the wing vein of chickens. It is possible to pierce a wing vein with a needle and collect the freely flowing blood into a small container. This delivers a sample that will probably be contaminated with bacteria and is less satisfactory than a sample collected using a syringe and needle. Also, the chicken is likely to be discoloured with blood, and some owners will object to this. The technique described below is less likely to stain the chicken with its blood and yields a better-quality blood sample. It based on the technique used by Dr Janeen Samuel (Australia) and Dr Rini Dharsana (Indonesia).

Materials needed

This technique uses a needle and syringe, both of which can be washed for reuse, though the needle less often than the syringe:

- a 25 G (0.50 × 16 mm) needle is used for chicks under 4 weeks of age
- a 23 G (0.65 × 32 mm) needle for older chickens.

Plastic syringes of 1.0 or 2.5 mL capacity are convenient.

If you have an assistant:

1. Ask the assistant to hold the chicken horizontally against their body with its head to their right.
2. Pull the right wing out towards you, pluck away the small feathers from the underside of the wing overlying the humerus, if necessary, and swab with 70% alcohol. The wing vein, named in various textbooks as the brachial, ulnar or cutaneous ulnar vein, is clearly visible running between the biceps and triceps muscles.

If working alone:

1. Sit with the chicken held horizontally between your thighs, head away from you, lying half on its back and half turned on its right side. (Some people prefer to hold the bird with its head towards them.)
2. Clamp down its legs with your left elbow (if you are right-handed) and its neck with your left forearm, and with your left hand spread out its left wing.

3. Insert the needle under the tendon of the pronator muscle, in the triangle formed where the wing vein bifurcates (see Figure A3.1), pointing the needle in the direction of the blood flow. Do not insert the needle too deep or it will scrape the humerus and the chicken will struggle. Also, avoid the ulnar nerve. With a little gentle probing you should enter the vein easily. (This approach from under the tendon makes it easier to enter the vein than does aiming directly for it, and also tends to steady the needle if the bird moves.)
4. Use only gentle suction to withdraw blood since the veins on chickens collapse readily. Collect 1–2 mL blood per chicken.
5. After the needle is removed, apply pressure to the vein for a few seconds to discourage further bleeding. Immediately cap the needle to prevent needle-stick injuries.
6. Immediately label the syringe with the number of the chicken.
7. If the blood is for serum collection, leave it in the syringe and store the syringe in a slanting position, with the *capped* needle end pointing upwards. Leave an air space between the blood and the needle end of the syringe. If possible, leave the syringes in a warm room at 37 °C for 1 hour to assist coagulation.
8. Ensure that all sharp items (e.g. needles) and contaminated items are disposed of in a safe manner.

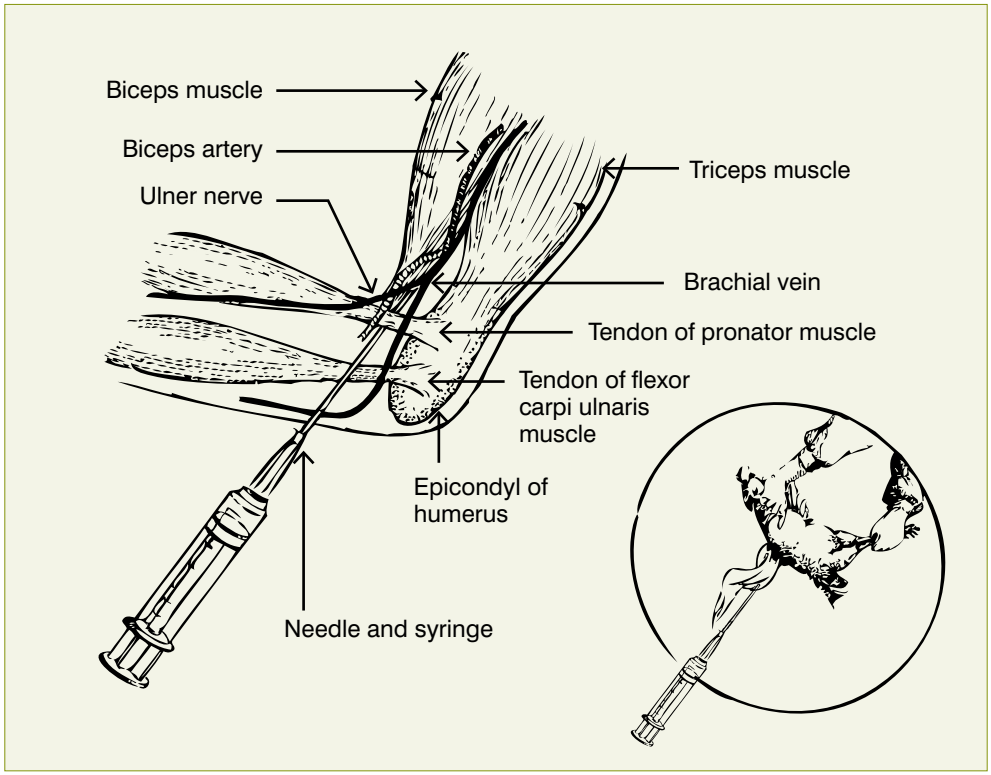

Figure A3.1 Illustration of a convenient anatomical site for bleeding chickens (adapted from J. Samuel, Virology Laboratory, University of Queensland)

Appendix 4

Post-mortem examination

A4.1 General information

When conducting a post-mortem, always wear appropriate protective clothing—gloves, waterproof apron, rubber boots and coveralls (overalls). If you suspect that the bird may have died of a zoonotic disease, a face mask (type N95) and goggles should be worn.

Clinical signs of sick chickens are often general (e.g. weakness, drooping wings, inappetence), or similar for several diseases (e.g. diarrhoea, respiratory distress). A post-mortem ('after death') examination reveals the organs affected by the disease and may also show characteristic lesions. Thus, post-mortem examination of a bird that died or has been killed after being sick strengthens and verifies a diagnosis based on case history and clinical examination. In this way, diagnosis of poultry diseases at field level can be enhanced with only limited financial input.

In general, post-mortem examinations are best performed in diagnostic laboratories. However, conditions in the field (long distances and lack of transport and cooling facilities) often make it impossible to send birds to the laboratory. Therefore, an extension officer should be able to confirm their diagnosis by performing a post-mortem in the field.

> **Note:**
>
> If there is a possibility that the bird to be examined has been infected with a zoonotic disease such as HPAI, it is essential that the technician use personal protective equipment (including gloves, a fitted N95 mask, safety goggles and coveralls).

Requirements for post-mortems performed under field conditions:

- **Materials needed**
  - a tray or a thick layer of clean paper (to perform the post-mortem on)
  - a sharp knife (to cut the muscles and skin). A stainless steel knife with a plastic handle is best. Ensure that it can be cleaned easily.
  - a pair of scissors (to cut muscles and skin and to open the intestines). Curved surgical (Mayo) scissors with both points rounded are best.
  - a pair of shears or garden secateurs (to cut the bones). A cheap, heavy pair of ordinary kitchen-type scissors is useful for cutting bones. If at least one blade is sharp-pointed, these scissors can be used for exposing the brain. For larger birds, ordinary garden pruning shears are ideal.

– a pair of forceps (rat-toothed are best; to hold any part of the carcass). If ordinary non-toothed forceps are used to handle organs and tissues, excessive handling and squeezing may damage the tissues. If fingers are used, the dissection field may become smeared with blood, which obscures detail. Fingers also tend to cause more bacterial contamination of exposed surfaces.

These materials should be washed and disinfected after each post-mortem. If disinfectant is not available, boiling water may be used instead.

If samples are to be sent to a laboratory for further diagnosis:

– bottles containing 50% glycerine in saline
– bottles containing 10% formalin *(The volume of 10% formalin used should be at least 10 times that of the tissue to be fixed.)*
– a cool box with ice or ice-packs (if possible).

Glycerine in saline is used to preserve samples for virus isolation. Formalin is used to preserve and fix samples for histological examination.

- **Where to perform the post-mortem**

  The post-mortem should be performed in dry, clean surroundings with as little dust and wind as possible.

  It should be performed on an even surface that:

  – is big enough to spread out the carcass and the organs (40 cm × 30 cm)
  – can be cleaned properly afterwards or will be discarded
  – does not allow the ground to be contaminated with any liquid (blood, faeces, intestinal content) or small parts from the carcass. (Sick chickens may carry and spread infectious agents.)

- **Selection of birds**

  When selecting birds for post-mortem, it is best to choose live birds that are showing typical signs, rather than dead birds or those that have been sick for some time. In dead or chronically ill birds, the primary disease may be obscured by secondary diseases or by post-mortem decomposition.

  If you are investigating a flock problem, then it is important to examine more than one sick bird at necropsy. For example, if three birds were examined, the accuracy of the subsequent diagnosis would be several times more likely to be accurate.

- **Disposal of the carcass**

  After doing a post-mortem the remaining carcass and all parts of the bird (including the feathers) should be buried or burnt to avoid further spread of infectious agents.

A4.2 Humane slaughter of birds

It is important to kill birds in a humane, efficient manner that does not itself cause changes that might confuse the diagnosis. Three techniques are described in this section. The first, cervical dislocation, is the most practicable under village conditions. Note that each of the techniques prevents the contamination of the environment with blood during the slaughtering process.

Examine the bird for clinical abnormalities before it is killed. This may indicate a particular system or organ that needs special attention during the post-mortem examination.

Cervical dislocation

1. Grasp the legs and primary wing feathers with one hand, so the bird cannot flutter.
2. With the other hand, grasp the bird's head from above, holding the head between the first (index) and second fingers. Curve the fingers along the bottom of the jaw. This avoids pressure on the larynx and tongue when the neck is broken.
3. Hold the bird across your body, with its head downwards. No undue force is used at any time up to this point. Bend the bird's head backwards.
4. Break the bird's neck using a fairly strong, rapid stretching action, keeping the head bent backwards. The bird will lose consciousness immediately, but will make strong reflex movements for about 2 minutes after neck dislocation. While struggling continues, keep the bird immobilised by maintaining your grip on the wing bases. Elevate the head to lessen the likelihood of inhalation of crop content, which may be regurgitated.

Intravenous injection of air

1. Grasp the bird by the wing bases with the left hand and immobilise it over the edge of a table.
2. Pluck a few feathers over the brachial vein.
3. Compress the base of the wing with the left index finger to distend the brachial vein.
4. Place the needle into the vein and rapidly inject 6–7 mL of air. Reflex struggling is brief and there is no trauma to neck structures. The bird does not regurgitate, as it may after cervical dislocation. Again, the wing bases should be held firmly until struggling stops.

Burdizzo

The burdizzo, a pinching tool used in the castration of cattle, can also be used to break the necks of poultry.

1. Two people must work together to use this technique.
2. One person holds the bird by the legs and primary wing feathers.
3. The second person places the burdizzo over the neck of the bird and closes them quickly to crush the vertebrae.

A4.3 Post-mortem technique for domestic fowl

There are many protocols for doing a necropsy on a bird. One protocol is described here. Whatever protocol you choose to follow, it should be one that is thorough and systematic, and that you feel comfortable with.

Work safely and cleanly

Ensure that water and soap are available for hand washing.

Collect cloacal and tracheal swabs before you begin the necropsy.

To avoid unnecessary contamination, collect samples for laboratory diagnosis DURING the post-mortem, not afterwards.

Examination procedure

A good necropsy involves careful external and internal examination of the carcass.

1. Examine the whole body, vent, breast muscle, skin, feathers, comb and wattles, nostrils, eyes and beak.
2. Spray or dip the carcass in a dilute solution of detergent or disinfectant to wet the feathers and reduce the risk of aerosolising infectious particles.
3. Position the carcass on its back with the legs towards you. Strip the skin from the breast, neck and legs and dislocate the hip joints by bending both legs outwards so that the carcass lies flat.
4. Open the abdomen and chest cavity. Avoid damaging any structures inside the carcass.
5. Examine the position and general appearance of the organs.

This is the '**display stage**' of the necropsy and is critically important. It is the last time you will be able to observe the organs in situ in an undisturbed, uncontaminated state. Any abnormal proportions, positions and configurations of organs must be noted at this time. Any accumulations of fluid or fibrin are noted now, as is the overall state of nutrition, presence or absence of overall carcass discolouration etc. Most of these features will be lost or hidden once the necropsy proceeds.

6. Examine the **air sacs, heart** and pericardial sac. Remove the heart and cut it open.
7. Pull the proventriculus, gizzard and small intestine to your left. Examine the **abdominal cavity**.
8. Cut the oesophagus at the junction with the proventriculus and free the gizzard and liver from their attachments. Cut the colon just before it enters the cloaca. Gently lift the intestines and cut their connection to the gastrointestinal tract close to the gut. Take the whole bowel (proventriculus, gizzard and intestines with liver and spleen) out to the left.

9. Cut the spleen from the bowel and inspect the **spleen**.
10. Cut the **liver** from the bowel. Inspect the liver and note the size of the **gall bladder**.
11. Examine the **testes** in roosters, and the ovary, oviduct and uterus in hens.
12. Loosen the **lungs** from the ribs and roof of the thorax and examine them.
13. Remove the ovary and remains of the air sacs to reveal the **kidneys**. Note their size, shape and colour. Check the small tubes connecting the kidneys with the cloaca (**ureters**).
14. Examine the **nerves**—the sciatic nerve plexus, the sciatic nerve, the intercostal nerves (between the ribs), the brachial plexus and the spinal and vagus nerves in the neck.
15. In young birds, open the **bursa of Fabricius** through its opening to the cloaca and examine.
16. Examine the **leg joints** and open them.
17. Cut off the tip of the beak just above the nostrils with shears to open the **nasal cavities** and **sinuses**. Open and examine them for changes.
18. Open the beak, larynx and **trachea**. Spread the trachea and examine it for liquid or other content and changes in colour.
19. Starting at the **oesophagus**, open the entire intestinal tract (**crop**, **gizzard**, **proventriculus**, **intestine** including the **caecum**).
20. Remove the head and gently open the **cranium**.

Finishing up

Carcass disposal

Dispose of the carcass immediately after finishing the necropsy. Proper carcass disposal will prevent the spread of the disease agent to other animals or humans through environmental contamination.

Guidelines for effective carcass disposal are given in Chapter 9.

Cleaning up

All objects that have come in contact with potentially infectious materials should be cleaned and decontaminated immediately after the necropsy. This will prevent the spread of the disease agent to other animals or humans.

Sterilise instruments between each necropsy by immersing them in alcohol and flaming them, or by leaving them in an acceptable disinfectant for the prescribed time prior to thorough rinsing in sterile water.

Particular attention must be paid to reusable equipment, instruments, vehicles, the environment and your hands.

Ensure that you have water, a wash bucket, nail brush, soap, paper towels and spray disinfectant with you.

Samples

All samples should be marked with the date and a unique identification number. Use only one identification number per animal, even if you are collecting several samples from the animal.

Data recording

Complete a detailed necropsy report to document your observations. Also complete a sample submission form and list the samples that you have collected.

A4.4 Common post-mortem lesions in chickens

Table A4.1 provides basic information on post-mortem lesions and their possible cause. The list is not complete and aims to help persons with little experience perform post-mortems on chickens.

Table A4.1 Signs that can be found doing a post-mortem on a chicken (listed in order of examination during the post-mortem examination)

Affected part of the body	Lesion	Possible cause
Muscles	Small haemorrhages (blood spots)	Infectious bursal disease (IBD)/Gumboro disease or septicaemia
	– pale	Infestation with blood-sucking parasites Loss of blood due to injury Rupture of the liver (fatty liver degeneration) Metabolic disorder
	– dark	Death after high fever
Air sacs	Cloudy	Chronic respiratory disease (CRD), infectious bronchitis (IB), colisepticaemia
	Pale mucoid thickened	Any chronic respiratory disease
Heart	Covered by cheesy material	Fowl cholera, fowl typhoid, colisepticaemia
	Covered with chalk-like material	Gout
	Fluid in the sac covering the heart	Fowl cholera, any disease affecting the circulatory system, nephritis
	Yellowish colour	Metabolic disorder, intoxication
	Small haemorrhages	Newcastle disease (ND), fowl cholera, highly pathogenic avian influenza (HPAI), other acute viral or bacterial infections
	Small white spots	Fowl typhoid, pullorum disease, Marek's disease, coligranuloma, tuberculosis
	Froth in the right atrium	The bird was killed by an intravenous injection of air.

Table A4.1 (Continued)

Affected part of the body	Lesion	Possible cause
Abdominal cavity	Cheesy content	Fowl cholera, colisepticaemia, fowl typhoid
	Liquid content	Fowl cholera, any heart or liver disease, intoxication
	Persistent yolk sac	Pullorum disease
	Tumours in or on various organs	Marek's disease, leucosis, various tumours
Spleen	Pale	Fowl typhoid
	Swelling	Fowl typhoid, fowl cholera, Marek's disease, leucosis
	Small haemorrhages	Fowl typhoid
	Small white spots	Tuberculosis, coligranuloma
Liver	Swelling	Fowl typhoid, fowl cholera, Marek's disease, leucosis, colisepticaemia
	Covered with cheesy material	Fowl cholera, fowl typhoid, infection with *Escherichia coli*
	Covered with chalk-like material	Gout
	Small white spots	Fowl typhoid, pullorum disease, Marek's disease, leucosis, coligranuloma, tuberculosis
	Pale yellow colour	Fatty liver degeneration
	Small haemorrhages	Fowl cholera, fowl typhoid
	Round lesions surrounded by haemorrhages	Histomoniasis (parasitic disease)
Ovary	Bloody	Acute infection, intoxication, ND
	Semi-solid ova	Bacterial infection
	Misshapen, discoloured ova	The hen has recently stopped laying eggs. Fowl typhoid, tumours, Marek's disease
Oviduct	Enlarged with cheesy content	Inflammation after bacterial infection
Lungs	Small white spots	Fowl typhoid, pullorum disease
	Small haemorrhages	Fowl cholera
	Cheesy content	CRD
	Foamy content	IB
Kidneys	Pale	Fowl typhoid, fatty liver degeneration, IB
	Swelling	Fowl typhoid
	Small haemorrhages	Fowl typhoid
Nerves	Thickened, greyish	Marek's disease

Table A4.1 (Continued)

Affected part of the body	Lesion	Possible cause
Bursa of Fabricius	Abnormal in size (enlarged or too small)	IBD
	Small haemorrhages	ND, IBD/Gumboro
	Cheesy content	IBD/Gumboro
	Small tumours	Leucosis
Trachea	Small haemorrhages	ND, laryngotracheitis, gapeworms
	Red worms	Gapeworms
	Yellow–white lesions	Fowl pox
	Mucous content	ND, IB
	Cheesy content	Infectious laryngotracheitis (ILT), IB
	Bloody content	ILT
Oesophagus	Yellow–white lesions	Fowl pox
Proventriculus	Small haemorrhages	ND, HPAI, intoxication, gout
	Blisters	Infestation with flukes
Small intestine	Ballooning	Coccidiosis, bacterial infection
Large intestine	Nodules	Tuberculosis, cancer of the pancreas
	Small haemorrhages	Coccidiosis, fowl typhoid, fowl cholera, necrotic enteritis (bacterial disease), ND
	Small stripes or spots	Coccidiosis
	Foamy content	Coccidiosis, fowl typhoid
	Mucous content	Tapeworms
	Cheesy content	Coccidiosis
	Worms	Tapeworms, roundworms, hairworms
Caecum	Bloody content	Coccidiosis
	Cheesy content	Chronic coccidiosis, histomoniasis (parasitic disease)

Note: Further information on selected diseases is given in Chapter 11.

Appendix 5

Collection of samples for laboratory examination

A5.1 General information

To identify or confirm the cause of a disease, examination of selected organs or tissues in the laboratory may be necessary. A confirmed diagnosis is especially important if ND or HPAI is suspected, since these are devastating and therefore notifiable diseases.

The diagnosis of an infectious disease can be confirmed by isolating the causative agent from affected organs and identifying it. Further laboratory examination may also be required to confirm diagnoses of diseases or conditions related to poor husbandry or nutrition, or to identify inherited conditions.

For best results, samples should be examined as soon as possible after collection. There are two options: sick chickens could be sent to the laboratory (NOT advisable if an outbreak of ND or HPAI is suspected because other flocks could be infected along the way) or samples could be collected at post-mortem in the field and conserved before submission to the laboratory.

When taking samples during a post-mortem:

- collect samples from sick chickens (or when no sick chickens are available, from birds that died recently)
- make sure that the instruments used to perform the post-mortem are disinfected, or at least cleaned with hot water
- make sure that the organs chosen for laboratory examination are not contaminated with dust or dirt during the post-mortem; handle organs using forceps
- submit several samples if possible, because this allows a wider range of examinations
- label all samples clearly.

Further details about the selection of samples for laboratory diagnosis of ND are given below. Information on selection of samples for other diseases is given in Section A5.5. Information on transport of samples and information that should accompany the samples is shown in Section A5.4.

A5.2 Samples for laboratory diagnosis of Newcastle disease

Samples should be collected from cases of suspected ND for virus isolation and identification. Fresh or conserved samples are suitable.

If samples will reach the laboratory within 24 hours, the **entire head** and samples of **spleen** and **lung** should be put in separate small plastic bags or leak-proof containers and kept cool.

If samples may not reach the laboratory within 24 hours, the **entire head**, the **long bones**, and samples of **spleen** and **lung** should be **conserved in 50% glycerine in saline**. These samples must also be kept cool.

A5.3 Samples for diagnosis of highly pathogenic avian influenza

Rapid test for influenza A

If HPAI is suspected, a rapid test for the detection of influenza A virus can be used by trained operators. Rapid on-site tests based on chromatographic immunoassay using rapid immuno-migration technology have performed best under field conditions.

A positive result with a chromatographic immunoassay test is usually a clear indication that the bird is infected with an influenza A virus. A false negative result may occur in approximately one in five cases truly positive for influenza A (false positives can be expected in up to 5% of uninfected chickens tested). Therefore, it is possible that the test will not detect up to approximately 35% of infected chickens. The test performs best (at the upper end of the ranges stated) when used to confirm a clinical suspicion of HPAI in live chickens that are severely ill or in birds that are not long dead, and it is for this purpose alone that it should be used. It does not perform well with birds that are incubating an infection.

When the test result is positive, the national HPAI contingency plan should be implemented immediately. When the test result is negative, samples should still be dispatched for laboratory testing, the area quarantined, and carcasses and contaminated material buried until the laboratory results are available.

Samples for virus isolation must always be collected

The use of the rapid test is NOT a substitute for the collection of specimens for virus isolation. Where HPAI is suspected, laboratory specimens must always be submitted for confirmation of the diagnosis and typing of the virus involved.

It is important that this kit be stored at 2–30 °C. It is best kept in the refrigerator (i.e. at 2–8 °C). DO NOT FREEZE the kit. It must not be stored in direct sunlight. If correctly stored, the kit can be used until the expiry date marked on the package label.

Tissue samples and swabs

Specimens should be collected from at least six birds. Preferably, three should be birds showing signs of the acute disease and the other three may be recently dead. The sick birds should be humanely slaughtered by cervical dislocation by hand or using a burdizzo.

If swabs are available, tracheal or oro-pharyngeal[3] swabs should be collected and placed in virus transport media.

If swabs are not available then the following tissues should be collected, placed in a sealed plastic bag and stored at 4 °C: spleen, lungs, air sacs, trachea, heart, pancreas, liver and kidneys.

If contamination is a problem, or storage conditions not ideal, then the whole head and a long bone should be placed in a sealed plastic bag and placed on ice.

These specimens should be transported in a secure container to the relevant veterinary laboratory. If specialised transport containers are not available, then a used, clean paint tin can be used. The plastic bag holding the specimens should be placed inside the tin and the tin placed inside a cool box containing pre-frozen ice packs.

Before reaching the international reference laboratory, the specimens should be stored and transported at 4 °C. The specimens must reach the laboratory as quickly as possible, as the virus may be inactivated after 4–7 days.

The specimens should NOT be frozen at –20 °C (i.e. the normal temperature in domestic freezers is –10 to –20 °C and so they are NOT suitable for storing samples destined for virus isolation). Repeated freezing and thawing must be avoided to prevent loss of infectivity. In the laboratory, the specimens will be frozen at –80 °C.

Transporting the virus on dry ice (i.e. frozen carbon dioxide) is not recommended as: (1) the dry ice may evaporate during the journey causing the specimen to thaw; (2) according to the International Air Travel Association regulations that cover the transport of goods on planes, dry ice cannot be placed in airtight containers; and (3) carbon dioxide can rapidly inactivate influenza viruses if it gains access to the specimens through shrinkage of tubes during freezing.

Serology

Blood samples should be collected for serum if there are suspicions of HPAI having occurred in the recent past, selecting in particular any birds that are known to have recovered from a respiratory disease. At the start

3 Tracheal swabs can be collected from dead birds. In live birds, it is more likely that samples will be collected from the oro-pharyngeal region (i.e. the throat area) as the birds will struggle to prevent the entry of the swab into the trachea.

of an outbreak, given the rapidity of spread of the disease in flocks, antibodies will rarely be present. The high fatality rate also leaves little opportunity for the virus to leave an antibody 'footprint' in the flock. Thus, serological tests do not assist greatly in the confirmation of HPAI A (H5N1) infections in chickens but can provide useful information in the case of ducks and geese.

Serological investigation in flocks vaccinated with homologous inactivated vaccines (i.e. H5N1, the same strain that is responsible for the outbreak) can be used to confirm that vaccination has stimulated an adequate immune response but not to test for the presence of disease. The use of heterologous vaccine (e.g. H5N2) will also affect serological investigations when the tests available can determine only the H subtype and not the N type of the virus.

Serum samples should also be collected from pigs, especially if they are showing signs of respiratory disease.

Sera should be separated from the clot as soon as possible in a clean area, taking care to transfer the reference details of each sample to the new tube. Sera may be stored at 4 °C for approximately 1 week, but thereafter should be frozen at –20 °C.

Good records and correct documentation are essential

When collecting samples, always record the sample number and case details (such as the village name, global positioning system (GPS) coordinates, species, history etc.). This will make follow-up communication with farmers and further investigations much easier.

A5.4 Sending samples to the laboratory

Organs will decompose at ambient temperature and infectious agents they contain may be destroyed. It is therefore very important that samples be kept cool until they reach the diagnostic laboratory. Pack samples in a cool box with sufficient ice or freezer bricks to keep them cold until they arrive at the laboratory. (Only samples conserved in 10% formalin for histological examination may be stored at ambient temperature.)

All specimens should be packed to prevent leakage, risk of accidental exposure of personnel handling the container, contamination of the sample or damage with water.

The cool box containing the samples should be clearly identified and accompanied by the following information:

- the name and address of the person sending the samples
- the date and location of sample collection

- case details—age, sex, breed, vaccination and treatment history, clinical signs, mortality and description of the outbreak
- differential diagnosis.

Central laboratories will usually have submission forms to record this information. It is advisable to have copies of submission forms available in field offices.

A simple and inexpensive container for the transport of hazardous biological specimens is described by Blacksell et al. (2006).

A5.5 Specific collection details

A5.5.1 Samples for diagnosis of viral diseases

Which samples can be used?

Virus isolation can be attempted from affected organs, which should be sent whole. Tissues, faeces and samples taken from any body fluid or moist surface using a sterile cotton swab can also be used for virus isolation.

Collecting the samples

Specimens should be collected by aseptic techniques, using sterile instruments and sterile containers. If this is not possible, try to work as cleanly as possible using instruments and containers that have been boiled shortly before use. Contamination of the specimen from other organs and tissues, and the environment (e.g. dust, dirt, feathers) must be avoided.

How to store the samples

- **Swabs**
  Swabs should be transferred to a transport medium immediately after collection. Suitable media should preferably be obtained in advance from the laboratory to which the samples are to be submitted. Swabs contain fresh samples and must therefore be sent to the laboratory immediately.
- **Fresh samples**
  Organs should be wrapped in plastic or placed in small bottles. Wrap or pack each organ separately. All fresh samples (organs, tissues, faeces and swabs) must be placed in a cool box with ice or icepacks soon after collection and submitted to the laboratory immediately.
- **Conserved samples**
  Where it is not possible to keep the samples cold, or when it is not certain that samples will arrive at the laboratory within 24 hours, organs or tissues can be conserved in 50% glycerine (glycerol) in saline and should be kept as cold as possible during dispatch. In the laboratory, the samples will be processed to isolate the suspected virus.

A5.5.2 Samples for diagnosis of diseases caused by bacteria or mycoplasmas

Which samples can be used?

Bacteria can be isolated from affected organs or fluids inside body cavities. Whole affected organs and samples of fluids or from moist surfaces, taken using sterile cotton swabs, should be sent to the laboratory. The swab is dipped into the fluid or stroked on the surface and then placed in a suitable transport medium.

For diagnosis of enteric bacterial diseases, intestinal contents, rectal swabs and smears of intestinal mucosa and pathological lesions can be used. To take a smear, firmly press a clean glass microscope slide onto the suspect area.

Collecting the samples

Specimens should be collected by aseptic techniques, using sterile instruments and containers. If this is not possible, try to work as cleanly as possible using instruments and containers that have been boiled shortly before use. Contamination of the specimen from other organs and tissues, and the environment (e.g. dust, dirt, feathers), must be avoided.

If fresh organs are being submitted for bacteriological examination, at the time of collection a small block of the organ should be fixed in 10% formalin for histopathology.

How to store the samples

- **Swabs**
  Swabs should be transferred to a suitable transport medium immediately after collection. Suitable media should preferably be obtained in advance from the laboratory to which the samples are to be submitted. Swabs contain fresh samples and must therefore be sent to the laboratory immediately.
- **Fresh samples**
  Organs should be wrapped in plastic or placed in small bottles. Wrap or pack each organ separately. Organs, and tubes containing transport medium with swabs, must be placed into a cool box with sufficient ice or icepacks to keep the samples cool until arrival at the laboratory. Samples must be submitted to the laboratory immediately.
- **Conserved samples**
  In general, all samples for bacteriological examination, except smears, should be kept chilled but not frozen, from the time of collection until they have arrived at the laboratory. Isolation of bacteria is not possible from conserved samples.
- **Smears**
  Smears are air-dried and wrapped separately for transportation.

A5.5.3 Samples for diagnosis of fungal diseases

Which samples can be used?

Any organs showing lesions can be submitted.

Collecting the samples

Specimens should be collected by aseptic techniques, using sterile instruments and containers. If this is not possible, try to work as cleanly as possible using instruments and containers that have been boiled shortly before use. Contamination of the specimen from other organs and tissues, and the environment (e.g. dust, dirt, feathers), must be avoided.

Small sections of affected organs should be conserved in 10% formalin for histopathological examination.

How to store the samples

Fresh organs for diagnosis of fungal diseases must be stored and transported chilled.

A5.5.4 Samples for diagnosis of parasitic diseases

Which samples can be used?

- **External parasites**
  Single external parasites (if it is possible to catch them) or infected feathers can be collected from the bird or their nests or roosts for identification.

  Scaly leg mites may be isolated by scraping the skin and scales of the birds' legs. (Since the lesions are characteristic for this parasite, laboratory confirmation of the diagnosis is rarely necessary.)
- **Worms**
  Worm eggs can be found in faecal samples. Fresh droppings from several birds can be collected and pooled (put together).

  Adult worms found in the intestines can be submitted to a laboratory for identification.

  Intestinal contents can also be submitted if infestation with worms is suspected.
- **Coccidia**
  Coccidia oocysts can be found in faecal samples. Fresh droppings from several birds can be collected and pooled (put together).

  Coccidia can also be found in smears or soft scrapings of intestinal mucosa. The mucosa of several sections of the small intestine and the caecum should be scraped with cover slips that are then put on slides.

Collecting the samples

If faecal samples are to be examined it is best to collect fresh droppings from several birds.

For isolation of scaly leg mites, scrape lesions on the chickens' legs using a knife moistened with paraffin.

How to store the samples

- **External parasites, adult worms**
  External parasites and adult worms can be collected in small leak-proof vessels and preserved in 10–15% KOH (potassium hydroxide) or 70% alcohol. External parasites found on the skin and feathers can be stored for some time before examination without conservation. Scrapings for examination of scaly leg mites can be submitted unpreserved in a small container.
- **Faecal samples, intestinal content**
  Faecal samples and intestinal content should be kept cool until they reach the laboratory.
- **Smears**
  Smears of intestinal content or mucosa should be kept cool until they reach the laboratory.

A5.5.5 Samples for histopathological examination

Histopathological examination of affected organs provides more information about the chicken's reaction to agents that cause disease, and may assist in identification of the cause of a disease.

Which samples can be used?

For histological examination, affected organs and tissues can be used.

Collecting the samples

Tissues collected at the port-mortem examination should be preserved immediately on collection (Table A5.1). Decomposed or frozen samples are unsuitable for histopathological examination.

It is important that only the edges of samples are handled so that histological detail is not lost.

- **Organs**
  Large organs or blocks do not fix adequately and are unsuitable for examination. In general, organs greater than 1 cm in thickness should be cut into 1 cm sections. Organs that are less than 1 cm thick can be fixed whole.
- **Nerves**
  Nerves commonly curl and twist when placed in fixative. Lay the nerve flat on a piece of card or stiff paper (which can be labelled) and allow to adhere for 3–5 minutes. The card with the attached nerve should then be immersed in fixative.
- **Oesophagus, intestines and other tissues**
  The section taken from any of these should include part of the lesion and the junction of the diseased portion with the apparently healthy tissue. To ensure adequate fixation, sections of unopened oesophagus or intestine should be immersed in fixative and gently pulled through the fixative a number of times to ensure that fixative enters the lumen.

How to store the samples

Samples should be fixed in 10% formalin. Always ensure that the tissue is preserved in an adequate volume of formalin: the volume of 10% formalin used should be at least 10 times that of the tissue to be fixed.

Samples fixed in 10% formalin can be stored for some time before examination, even at ambient temperatures.

Table A5.1 Organs or other specimens suitable for laboratory diagnosis of certain diseases or syndromes

Disease/syndrome	Specimen required
Chronic respiratory disease	Trachea, choanal (palatine) cleft
Coccidiosis	Faecal samples, intestinal content Swabs or soft scrapings from intestinal mucosa Portions of intestines showing lesions in formalin (for histopathology)
Colisepticaemia	Whole, fresh dead chicks Liver, kidney, lung Swab samples from the pericardial sac, air sacs and joints
External parasites	Parasites, infested feathers Scraped lesions of scaly legs
Fowl cholera	Liver, bone marrow *(recommended when specimens are not fresh or when contamination of tissues is likely)*, heart blood
Fowl plague, avian influenza	Cloacal and tracheal swabs Trachea, lungs, air sacs, exudate from the sinus
Fowl pox	Recently developed nodular lesions
Fowl typhoid	Liver, spleen, heart, ovary
Infectious bursal disease/ Gumboro disease	Bursa of Fabricius
Infectious bronchitis	Tracheal swabs, cloacal swabs Lungs, kidneys, oviduct, caecal tonsils *(Virus might be isolated from cloacal swabs or caecal tonsils even several weeks after clinical signs have disappeared.)*
Infectious coryza	Entire head Swabs from the sinus cavities *(Tracheal swabs do not always contain the causative bacterium.)*
Infectious laryngotracheitis	Tracheal swabs Trachea, lung
Marek's disease	Organs with tumours Nerves (for histopathology)
Newcastle disease	Spleen, lung, entire head, long bones
Pullorum disease	Whole, fresh dead chicks
Worms	Faecal samples, intestinal content

A5.6 Serology

Serological examinations provide valuable diagnostic information in poultry flocks. A variety of methods can be used to detect antibodies and antigens circulating in the chickens' blood for direct or indirect diagnosis of infectious agents.

This appendix does not provide information on techniques used for serological examination. It aims to assist with the interpretation of results.

Accurate interpretation of serological findings requires knowledge of the efficiency and reliability of the technique used (sensitivity and specificity), and information on the course of the disease and the impact of immune reactions caused by specific infectious diseases. High antibody titres do not necessarily indicate protection against a certain infectious disease: **Protection against infectious diseases is obtained by a complex cooperation of various components of a bird's immune system, antibodies found in the serum being only one of them**.

Optimal time for sampling

A serological reaction to any infection can be detected not earlier than 1 week after infection or vaccination. In general, blood samples are taken 2 weeks after vaccination when high antibody titres are expected.

Sampling size and selected birds

For an infectious agent that spreads quickly within a flock—like the ND or HPAI viruses—many positive results can be expected, while only a few positive results might be found for a slowly spreading infectious agent. Thus, the number of samples to be taken within a flock depends on the percentage of expected positive results and on the reliability aimed for the results.

In order to measure whether vaccination has been effective, birds could be sampled at a rate sufficient to determine whether 80% of birds had a protective titre, with an accuracy of ±10%. (Epidemiological theory suggests that if at least 70% of a population is immune then disease outbreaks are unlikely to occur because there are not enough susceptibles to propagate an epidemic. Determination of 80 ±10% allows an assessment to be made of whether or not more than 70% of the population have protective titres.) Table A5.2 lists the number of birds would then need to be sampled (sample sizes have been rounded up) according to flock size.

Table A5.2 Recommended number of birds to be sampled to determine if 80% of a flock has a protective titre after vaccination

Flock size	Sample size
100	40
200	60
300	55
400	55
500	60

When selecting birds for sampling, birds of all age groups should be included, not just chickens that are easy to catch. True random selection (giving every bird the chance to be sampled) provides more comprehensive information on the whole flock.

Positive results

Clearly positive serological results, even of single birds within a flock, indicate contact with the infectious agent—but not necessarily clinical disease caused by this agent (e.g. antibodies for IBD/Gumboro might be found in adult chickens, which do not get sick from this disease because IBD/Gumboro affects only young chickens). Furthermore, positive results do not reveal whether the infection occurred in the past or is still active. To get further information on the actual presence of an infectious agent, two samples taken within a certain time from the same bird have to be examined (see paired serum samples, below).

Negative results

Clearly negative results obtained from random samples, on the other hand, do not prove the absence of a particular infectious agent from the flock.

Paired serum samples

Examination of two samples per chicken with a certain time interval between the two samplings is known as examination of 'paired serum samples'. This method is used to get information on the time of infection. The time between the two samplings (2 weeks; Table A5.3) should be sufficient for antibody titre to develop.

Table A5.3 Interpretation of paired serological samples

Results		Interpretation
1st sampling	2nd sampling	
Negative	Positive	• A change from a negative to a positive result (antibody status) is known as seroconversion and indicates an acute primary infection.
Positive	Rise in titre	• A significant rise in antibody titre between early and late phase samples can indicate an active infection. (It does not reveal whether this is the bird's first infection with this agent.) • Rise in antibody titres could also result from booster vaccination or stress-induced reactivation of latent infection.
Positive	Fall in titre	• A fall in antibody titre between the two samplings indicates a previous infection that is no longer active. • A few weeks (2–4 weeks) after vaccination the antibody titre starts to decline continuously.
Negative	Negative	• If both examinations reveal negative results the bird had had no contact to the infectious agent at the time of 1st sampling. Nevertheless, recent infection (within the past week) might have happened without detectable antibodies being present at the time of 2nd sampling.

Thus, seroconversion is more convincing diagnostically than detection of a significant rise in titre.

Appendix 6

Sources of further information

More details on village poultry health and production may be found at the following websites:

- **International Rural Poultry Centre** at <http://www.kyeemafoundation.org/irpc.php>
- **International Network for Family Poultry Development** at <http://www.fao.org/ag/AGAinfo/themes/en/infpd/home.html>
- **Danish Network for Smallholder Poultry Development** at <http://www.poultry.kvl.dk>
- **Australian Centre for International Agricultural Research** at <http://www.aciar.gov.au>
- **Food and Agriculture Organization of the United Nations** at <http://www.fao.org>

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