EAZA Reference Document - Virus Management for Parrots

Is this a healthy parrot?

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Goal

The goal of these guidelines is to reduce the risks and impact of viruses to parrots in EAZA.

Introduction

Parrots are one of the most threatened bird orders with 28% of species listed as globally threatened and 56% of all parrot species in decline. The Parrot TAG has established breeding programmes for many threatened species and subspecies as insurance populations for their wild counterparts. These breeding programmes aim to create/maintain healthy populations of healthy individuals.

In order to ensure that our parrot programmes maintain healthy populations of healthy animals, the risks posed by major parrot viruses must be minimised. The production of these guidelines generated a lot of veterinary debate on how to best screen for the different viruses. We advise implementing a system with the highest level of biosecurity in order to minimise the risk of these viruses to your collections. Please share these guidelines with your vets and if there are any questions, please let us know.

Important notice for holders of parrots

Institutions holding parrots species or who wish to keep these species need to incorporate the costs of sampling and testing into their budgets for the best interest of these birds.

Institutions holding parrots which are part of a managed programme and test positive for one of these viruses, need to consult with the programme manager regarding the future management of these parrots. Holders and coordinators are called upon to seek pragmatic, inclusive and sensible solutions to achieve the best interest of the overall parrot populations and their ongoing sustainability.

Parrot viruses of concern

The main parrot viruses currently of concern are listed in this table:

Virus	Mortality Rate	Infection Rate
ABV = Avian Bornavirus = Avian Ganglioneuritis (formerly PDD (Proventricular Dilatation Disease) but this is just one symptom)	Moderate	Low
APV = Avian Polyomavirus	Moderate High in chicks	High
PBFD = Psittacine Beak and Feather Disease = Avian Circovirus	Moderate High in chicks	High
PsADV = Psittacine Adenovirus	Moderate but can be high in some species	Moderate
PsHV = Psittacine Herpesvirus = Pacheco's Disease	High	High

The prevalence of these viruses in our collections is largely unknown so an important first step is to obtain this information for each virus by analysing recent test results and encouraging greater levels of testing and sharing of results especially within EEPs.

It is important to note that testing is just one aspect of virus management. It is vital that testing is combined with good biosecurity protocols and good record-keeping – see "Reducing the risks" below for further details.

When to investigate the presence of viruses?

The Parrot TAG recommends the following instances when virus investigations should be carried out:

- Before **any** new parrot enters your collection (this can be pre-transfer and/or post-transfer but in isolation).
- If a parrot is showing clinical signs.
- All dead parrots (including chicks) should receive a full post-mortem (including histopathology and further lab diagnostic tests such as PCR) to investigate the prevalence of viruses and occurrences of dormant infections – even those with an obvious cause of death (e.g. trauma)
- On request of the EEP coordinator, when needed to make informed decisions about the overall population.

In addition to the above, testing in the following situations can add to our understanding of the prevalence of these viruses and their impacts:

- If a parrot seems to be producing infertile eggs/eggs which do not develop; an underlying illness may be the cause
- If a parrot needs to be euthanised, please collect blood for serology as this can be useful in determining if the parrot was ever in contact with these viruses in the past.
- Random testing – if a parrot needs to be handled, samples can be taken opportunistically to test for viruses.
- Random testing – eggs which do not hatch can be tested for Circovirus and Polyomavirus. These viruses are fairly resistant but eggs should be as fresh as possible.

If your institution holds breeding parrots which are part of a breeding programme, then all these parrots (and any parrots they are in contact with) should be tested to ensure these viruses are not already present.

Recommended virus tests

Briefly, PCR tests investigate the presence of the virus itself, while serological tests determine the host response to the virus. Please see the table below and accompanying notes underneath.

Virus	**PCR**	**Serology** (Serum preferable)
Avian Bornavirus	Cloacal swab and crop swab which can be pooled Brain from dead birds	Blood sample - 500 µl Li-He **or** 500 µl EDTA blood **or** 200 µl serum
Avian Polyomavirus	Growing feathers AND a whole EDTA* blood sample (200µl) should be taken. The blood sample should only be tested if the feather is negative [(1)] *with* • Bursa of Fabricius from dead chicks or • Feathers and spleen from dead adults	Blood sample - 500 µl Li-He **or** 500 µl EDTA blood **or** 200 µl serum
Avian Circovirus (PBFD)	Growing feathers AND a whole EDTA* blood sample (200µl) should be taken. The blood sample should only be tested if the feather is negative [(1)] *with* • Bursa of Fabricius from dead chicks, • feathers and spleen from dead adults	Not applicable

Psittacine Adenovirus	Cloacal swab or faecal sample (swab preferred) AND a whole EDTA* blood sample (200µl). The blood sample should only be tested if the cloacal swab/faecal sample is negative (1) (2) Liver and spleen from dead birds	Blood sample - 500 µl Li-He **or** 500 µl EDTA blood **or** 200 µl serum
Psittacine Herpesvirus (Pacheco's Disease)	Cloacal swab or faecal sample (swab preferred) AND a whole EDTA* blood sample (200µl). The blood sample should only be tested if the cloacal swab/faecal sample is negative (1) (2) Liver and spleen from dead birds	Blood sample - 500 µl Li-He **or** 500 µl EDTA blood **or** 200 µl serum

* EDTA is better but heparin blood samples will also work

Total blood needed to test for all viruses: 500µl serum or plasma (1.0ml EDTA) for all tests**.** However, the volume collected should not exceed 1% of the bodyweight (including the blood if there is bleeding after venepuncture and/or occurrence of haematoma). Parrots do not need to be anaesthetised for blood collection (see below for further information).

In smaller parrots (less than 100g), a drop of blood on a swab/filter paper can be used for PCR. However, for serology a real blood sample (serum or plasma) is required. Parrots weighing less than 100g can have smaller amounts of blood taken at separate instances and then can be submitted for individual tests for serology. If taking blood more than once, an interval of 7 days is recommended.

Alternatively, for the PCR-tests, if an institution has a flock of small parrots (weighing under 100g), a pooled sample from several individuals (maximum 3) in a flock can be tested. Pooled samples should never be used for serology as this can lead to incorrect test results.

(1) Send both samples to the laboratory. Good laboratories with an avian background will test just the feather sample (or cloacal swab/faecal sample in the case of Adenovirus and Herpesvirus) at first and only use the blood sample if the feather is negative. If both the feather and blood sample are pooled and tested together for a specific virus, this can result in loss of sensitivity. It is important that both samples are sent to the lab; the feather sample could be negative because the parrot very recently contracted the virus and therefore the blood sample is needed to confirm the status.

(2) This virus is only in the blood after it has been replicated in the liver and/or intestinal cells so the time it is in the blood before shedding is very short. If testing the parrot twice (4 – 6 weeks apart) for this virus, then a cloacal swab/faecal sample for both PCR tests will suffice.

PCR (Polymerase chain reaction)
PCR detects viral RNA or DNA and if positive confirms that a certain fragment typical for the virus is currently present in the sample. As this fragment is specific for the virus, it can be assumed that the virus is present at time of sampling. PCR cannot distinguish between live and dead virus.

Serology
Serology detects antibodies produced when the bird was or is in contact with the virus. The parrot may no longer have this virus or may be a carrier (depending on the virus).

Avian Bornavirus:
A dry cloacal swab and a crop swab in addition to serum are advised. The two swabs can be pooled for one PCR. Samples should be tested within 5 – 7 days of being taken and transported cool (no need to freeze).

Both PCR and serology are necessary as Avian Bornavirus has intermittent shedding (so not always possible to detect by PCR) and infected birds do not always seroconvert (so not always possible to detect via serology).

Avian Polyomavirus It is recommended to collect a few growing feathers for PCR testing in addition to blood.
Serology is especially important when testing for carrier birds.

Avian Circovirus (PBFD)
Growing feathers is the sample of choice for PCR. However, in a few cases, viral DNA might be found in the blood, with negative feathers. Therefore, with negative feather results, blood should be tested by PCR as well.
Antibody testing is difficult to interpret as seroconversion and clearance of the virus is not well understood. Therefore, serology is not recommended at this stage.

Psittacine Adenovirus
Cloacal swabs or faecal samples can be used for PCR or cell culture to grow the virus. Electron microscopy may also be used but it is complicated, not very sensitive and expensive. Adenovirus is only in the blood after it has been replicated in the liver and/or intestinal cells so the time it is in the blood before shedding is very short. If testing the parrot twice (4 – 6 weeks apart) for this virus, then a cloacal swab/faecal sample for both PCR tests will suffice.
Antibodies against adenovirus should be tested to detect seropositive carrier birds.

Psittacine Herpesvirus (Pacheco's Disease)
Cloacal swabs or faecal samples can be used for PCR or cell culture to grow the virus. Electron microscopy might also be used but is complicated, not very sensitive and expensive. Herpesvirus is only in the blood after it has been replicated in the liver and/or intestinal cells so the time it is in the blood before shedding is very short. If testing the parrot twice (4-6 weeks apart) for this virus, then a cloacal swab/faecal sample for both tests will suffice.
Antibodies against herpesvirus should be tested to detect seropositive carrier birds. Here it is important that the antibodies are tested against Psittacine herpesvirus-1 (there are several other psittacine herpesviruses so it is important to test specifically for PsHV-1).

Additional note on parrots new to your institution:
At a minimum, all new birds should be tested at least once for these viruses (either pre-export or post-import). Ideally, a second set of tests should be conducted 4-6 weeks after the transport. Transport and a new environment can result in higher levels of stress and silent virus infections can turn into active infections or silent shedders, which can lead to flock infections.

Note on vaccines
Even if a vaccine is available for a particular virus, there is currently no recommendation to vaccinate. The vaccine does not prevent infection with the field virus and so you still have carrier birds but cannot distinguish if they are positive for a field virus infection or vaccination.

Sampling technique and transport

Anaesthetic is not required to take these samples.

Proper sampling, storage and transport are important elements in obtaining accurate test results.

Taking swabs (eye, choana, cloaca):

Samples should be stored cool /2-5°C) for no longer than 7 days (including shipment to the laboratory). If frozen, they should reach the laboratory frozen; thawing and refreezing should be avoided.

Taking feather samples:

Select 3-5 growing feathers (growing feather with blood supply to shaft). Storing and shipment similar to swabs above. A different set of gloves and tweezers (if used) should be used for each bird to avoid contaminating the samples.

Taking blood:

The best place to take blood usually is the right jugular vein in the neck or the ulnar vein on the inner side of the wing. The limiting factor is that blood volume should not exceed 1% of the bodyweight. Enough blood can generally be collected using physical restraint of the parrot.

Transporting samples

Samples should be transported cool and arrive within 7 days if stored by 2-5°C

Choice of laboratory

Laboratories can vary from one another in a number of ways:

- They may be using different primers for the PCR tests
- They may be testing for different strains of the virus e.g. circovirus, avian bornavirus
- Different levels of security for accurate results may be used. Positive and negative controls increase the price of examination but are really important to ensure the quality of the results
- Different levels of human competencies

The laboratory you use should be accredited for the tests you have requested. Some countries have central agencies which register accredited laboratories. Good laboratories (those who are accredited) regularly participate in 'ring tests' and get spiked samples (i.e., known positives) to ensure their quality. This level of quality control (including positive and negative controls etc.) is cost intensive - so these labs are usually more expensive. However, the test results from these accredited laboratories are generally more reliable than from laboratories which have not undergone this accreditation process.

It is also important that the lab is specialised in birds in order to provide advice on what the test results mean for the flock and if re-testing etc. is recommended. If there are inconsistencies in the test results, a good lab specialised in birds should spot these and advise accordingly.

When choosing a laboratory, it is important to understand what is meant by test sensitivity, test specificity and test accuracy.

		True status	
		Status positive +	**Status negative -**
Test result/ Predicted status	**Positive**	True positive	False positive
	Negative	False negative	True negative

Test sensitivity: Proportion of birds with the virus who are correctly identified as being positive (true positive)

$$\text{Sensitivity} = \frac{\text{true positives}}{\text{(true positives + false negatives)}}$$

Test specificity: Proportion of birds who do not have the virus who are correctly identified as being negative (true negative)

$$\text{Specificity} = \frac{\text{true negatives}}{\text{(true negatives + false positives)}}$$

Ideally, both sensitivity and specificity are high, but sometimes if one is high the other is reduced. Accuracy reflects the sum of the two, the best compromise:

$$\text{Accuracy} = \frac{\text{(true positives + true negatives)}}{\text{(true positives + false positives + false negatives + true negatives)}}$$

Some laboratories provide test sensitivity, specificity and accuracy data on the tests they provide. If a laboratory is accredited for the test, then a high level of sensitivity and specificity should be present.

How to interpret results?

What do negative results mean?

- The parrot is negative for the virus
- The sample used (e.g. feather) is negative for the virus (but the parrot may still be positive)
- The parrot is not viraemic (i.e. the virus is not circulating in the blood)
- The sample was degraded
- The virus genotype tested for was not detected
- There was a lab error in reading the results

What do positives mean?

- The parrot is positive for the virus (however, when the parrot dies – always conduct a post-mortem to validate this result) – The pathologist should be told that there was a positive serology/PCR prior to conducting the post-mortem examination so they can search for signs.
- The parrot is negative but the sample was contaminated (most likely to happen with feathers)
- The test was contaminated in the lab
- There was a lab error in reading the results

Sometimes the PCR and serology yield different results:

PCR positive and serology negative: The infection is recent and antibodies have not had time to form or the immune system is compromised and is not producing antibodies to fight the virus. Another option is that

the virus is able to hide from the immune system so that antibodies are not developing during infection. It is also possible that there is a false positive PCR-result or a contaminated sample.

PCR negative and serology positive: The parrot has had contact with the virus; therefore, it has developed antibodies but is not currently shedding the virus. The parrot is either free from the virus (recovered) or a carrier (and may be shedding intermittently). It is also possible that it is a false negative PCR result or the sample was degraded.

The following factors should also be considered when interpreting results:

- Individual history – recently arrived, contact with parrots of unknown health status, outdoor aviary
- Whether there are clinical signs or not
- Flock status – testing history of the flock and contact with other parrots
- Post-mortem findings if the bird dies
- Contact with people who may also have contact with other parrots e.g. keepers who work with parrots in other parts of the institution, keepers who keep parrots at home, and walkthrough aviaries where private parrot holders may have close contact.
- Contact with wild parrots

Note on silent carrier parrot species

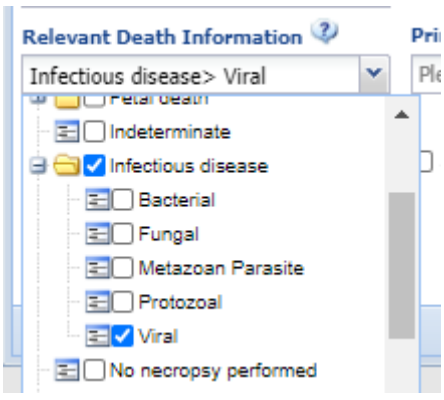
Some parrot species are more likely to be silent carriers of certain viruses; rarely develop clinical illness but intermittently shed the virus. For example, Quaker (monk) parakeets (*Myiopsitta monachus*) and Patagonian parakeets (*Cyanoliseus patagonus*) can be silent carriers of herpesvirus. Ideally these species should not be held next to species more vulnerable to these viruses and different keepers should work with them. However, it is important to note, that all parrot species are potential carriers of viruses and all should be tested appropriately.

Sharing results

Accurate record keeping is essential to understand the prevalence of these viruses in EAZA. The Parrot TAG requests that all results (positive and negative) are entered into ZIMS Medical using the "Tests and Results" tab.

Post-mortem results - Keeping records of post-mortem investigations are also important and should include information on whether signs of viral infections were looked for. Dead birds are the mirror of the flock - they should automatically be tested for the most common viral diseases, as organ samples are better than swabs to detect carrier birds. If the presence of one of these viruses is detected post-mortem, please record in ZIMS using the relevant tabs.

The Parrot TAG requests that, at a minimum, the cause of death is recorded in ZIMS Medical by completing the Relevant Death Information box in the "Necropsy" tab and if the presence of one of these viruses was detected post-mortem (using the "Tests and Results" tab). It is also recommended to send post-mortem results to EEP/ESB coordinators if the bird was part of a manged breeding programme.



If the cause of death is from a viral disease, then this should be recorded using the Relevant Death Information (RDI) field in the Necropsy module in ZIMS. When the RDI field opens initially, the infectious disease node is collapsed. When this node is expanded, then both infectious and viral can be selected – see opposite.
(Special thanks to Dr. Teare from Species360 for his advice on using the RDI field)

How to manage positive parrots?

Although these viruses are present in certain wild populations of parrots, they can cause major problems in naïve populations and smaller populations such as zoo breeding programmes. **All institutions housing parrots need to plan for the possibility of having to manage positive parrots.**

The best scenario would be to have virus-free breeding populations. However, many of the parrot breeding programmes are working with small populations and total exclusion of some animals may have negative consequences on the genetic health of the breeding programme. Institutions holding positive birds thus need to manage these birds appropriately to prevent the spread of the virus to other birds in their collections or to other institutions. Please see the paragraphs below for further information on managing specific viruses.

Retesting is especially important if there are no clinical signs to match the test results. If there are doubts regarding the competency of the lab you have been using, then changing laboratories needs to be considered.

If a parrot remains positive, there are a number of points to consider. **For species which are part of managed breeding programmes, it is important that their management be discussed with the coordinator. The welfare of the parrot should not be compromised.**

The Parrot TAG recommends having an agreement in place between institutions when transferring parrots on what to do in the event of a bird testing positive for a virus upon arrival in a new institution. This agreement should include notifying the coordinator if the parrot is in a managed programme.

The establishment of positive flocks may be beneficial in some cases, as for some viruses it is known that positive birds can produce negative offspring which means that the infected parents are still vital for the programme. However, positive birds always pose a risk so such flocks/pairs need to be separated from negative psittacines.

It may be of benefit for a small number of institutions to take all positive birds but it may be difficult to find such institutions. However, these viruses do not pose a risk to non-psittacine birds or other major taxa e.g. mammals, reptiles so institutions not holding other parrots may be suitable locations for positive flocks. It is important to note that it is not clear what the impact would be on a parrot carrying more than one of these viruses. Therefore, it is not practicable to mix parrots with different viruses as it could lead to super infections.

Below are some guidelines on how to manage parrots which are positive for a particular virus:

ABV = Avian Bornavirus = Avian Ganglioneuritis

ABV is not a highly contagious virus and does not survive long outside of the parrot's body. Parrots can live for many years with this virus and not show clinical signs. Parrots positive for this virus can also produce eggs/chicks which are negative. However, eggs should be either artificially incubated or hatched by foster parents and chicks hand-reared or reared by negative parrots.

Parrots which have tested positive for this virus should be housed together, kept separate from negative parrots and cared for by separate keepers. The traffic light system (Appendix I) can be used to clear a flock from Avian Bornavirus.

It may be possible to have two separate breeding populations; one for ABV positive birds and another for negative birds. However, all birds within that breeding programme would need to be tested in order to manage this effectively.

APV = Avian Polyomavirus

Avian Polyomavirus spreads easily between birds. Therefore, birds should be separated, tested individually and positive birds removed. In a positive flock, breeding should be ceased to allow the adults to produce antibodies. When breeding is taken up again -usually 1 year later- offspring are usually protected against deadly clinical disease. However, those birds are usually also carriers and should also not be mixed with negative birds.

Polyoma-positive flocks are of very limited use to a breeding programme. They might be used as exhibition or ambassador birds if not clinically affected, if their welfare can be assured and if there is no direct or indirect contact to negative parrots. However, as our knowledge grows about this virus, this recommendation may change.

Positive and negative flocks should not be cared for in the same physical location or with the same keeping teams. Positive flocks need to be managed in strict quarantine from negative parrots. It may be possible to move positive parrots to established holdings of polyoma positive flocks or holdings with no other parrots but it is important that good welfare can be assured. It is also important to discuss how to manage positive results with the Parrot TAG veterinary advisors. Euthanasia should only be considered as a last resort in clinically healthy parrots and after discussion with the Parrot TAG veterinary advisors.

PBFD = Psittacine Beak and Feather Disease = Avian Circovirus

Circovirus is highly contagious and can be spread via inhalation or ingestion of feather dust and faeces; it is also likely that it is transmitted into the eggs. It is very stable in the environment. Therefore, birds should be separated, tested individually and positive birds removed. Positive birds are usually also carriers and should also not be mixed with negative birds.

Circo-positive flocks are of very low use to a breeding programme. They might be used as exhibition or ambassador birds if not clinically affected, if their welfare can be assured, and if there is no direct or indirect contact to negative parrots. However, as our knowledge grows about this virus, this recommendation may change.

Positive and negative flocks should not be cared for in the same physical location or with the same keeping teams. Positive flocks need to be managed in strict quarantine from negative parrots. It may be possible to move positive parrots to established holdings of polyoma positive flocks or holdings with no other parrots but it is important that good welfare can be assured. It is also important to discuss how to manage positive results with the Parrot TAG veterinary advisors. Euthanasia should only be considered as a last resort in clinically healthy parrots and after discussion with the Parrot TAG veterinary advisors.

PsADV = Psittacine Adenovirus

This is a moderately to highly contagious virus (depending on species) which can spread directly and indirectly. Infected birds with symptoms should be quarantined and given intensive supportive care to help them survive (often severe symptoms with rapid onset). Other diseases should be suspected in positive parrots. There is a specific test for psittacine adenovirus but some laboratories are unable to differentiate between the different adenoviruses (there are different types of adenovirus and not every adenovirus is pathogenic for parrots).

Psittacine Adenovirus survives in a flock usually through a few carrier birds. Stress supports shedding and triggers an outbreak. Therefore, carrier birds need to be identified and removed from the flock. A positive flock may be able to produce negative offspring through artificial incubation.

PsHV = Psittacine Herpesvirus = Pacheco's Disease
Herpes virus is spread by direct contact, aerosol, or faecal contamination of food or water and is highly contagious. The virus is not stable in the environment and does not survive long outside the host; however, it can survive for weeks in faeces. Typical clinical signs are sudden death or death after a few hours of illness.
Positive but clinically healthy birds should be removed from the flock and maintained in strict quarantine. A positive flock may be able to produce negative offspring through artificial incubation.

Reducing the risk

- Know the infection status of your flock by testing all parrots.
- Always test new birds arriving into your institution.
- Incorporate opportunistic testing of parrots when they need to be handled (and unhatched eggs) into your institution's disease surveillance programme.
- Submit all dead birds to a full post-mortem including routine virus testing independent of cause of death or gross findings in the post mortem.
- Have experienced keepers working with the parrots who can spot clinical symptoms early.
- Keep accurate records of parrot observations and test results/post mortem findings to understand the prevalence of these viruses in your collection.
- Restrict contact between zoo parrots and wild parrots.
- Maintain high levels of biosecurity for keepers especially if in contact with other parrots species outside of your institution. Parrot keepers should be given biosecurity training to ensure they are not a vector for parrot viruses.
- All walk-through aviaries should be assumed as being potentially positive as these viruses may be brought into the aviary at any time by a visitor. The guidelines described below under "Biosecurity guidelines for housing positive parrots" should be followed.
- Parrots are more susceptible to contracting viruses if their immune system is compromised. Therefore, high standards of parrot husbandry are vital e.g. appropriate nutrition, regular cleaning of the aviaries, regular screening for faecal parasites and bacteria, minimising exposure to stressful situations and maintaining high animal welfare standards.
- If using an animal transporter to move your parrots, ensure that parrots from other institutions do not travel with them unless they have undergone the same level of pre-export testing.

Biosecurity guidelines for housing positive parrots

If housing parrots which have tested positive for one of the above-mentioned viruses, it is important that these viruses do not spread to other parrots in your institution. Maintaining positive and negative flocks within the one institution is possible for Avian Bornavirus but more challenging for Herpesvirus and Adenovirus. It is not possible with circovirus and polyomavirus.

The following biosecurity guidelines are recommended:

- Separate keepers for positive parrots and negative parrots.
- Separate tools/equipment and food preparation areas.
- Positive parrots should be housed in a separate part of the zoo from negative parrots. Parrots positive for a virus which can be spread through the air should be housed indoors.

- Footbaths/shoe protectors/epidemic carpets should be used to prevent keepers transmitting the virus on their shoes.
- Use gloves and proper hand-washing facilities.

Research on parrot viruses

If institutions are interested in supporting research activities on parrot viruses, please get in touch with the Parrot TAG or any of the TAG veterinary advisors.

Current topics of interest to the Parrot TAG include:

- Investigating the prevalence of these viruses in EAZA institutions. Research on prevalence and the level of testing would be greatly aided if institutions record testing and results as described in the section above on sharing results.
- Investigating the prevalence of these viruses in feral parrot populations living in EAZA member locations.
- Further research on vertical transmission and the possibility of generating negative offspring from positive parrots/flocks.

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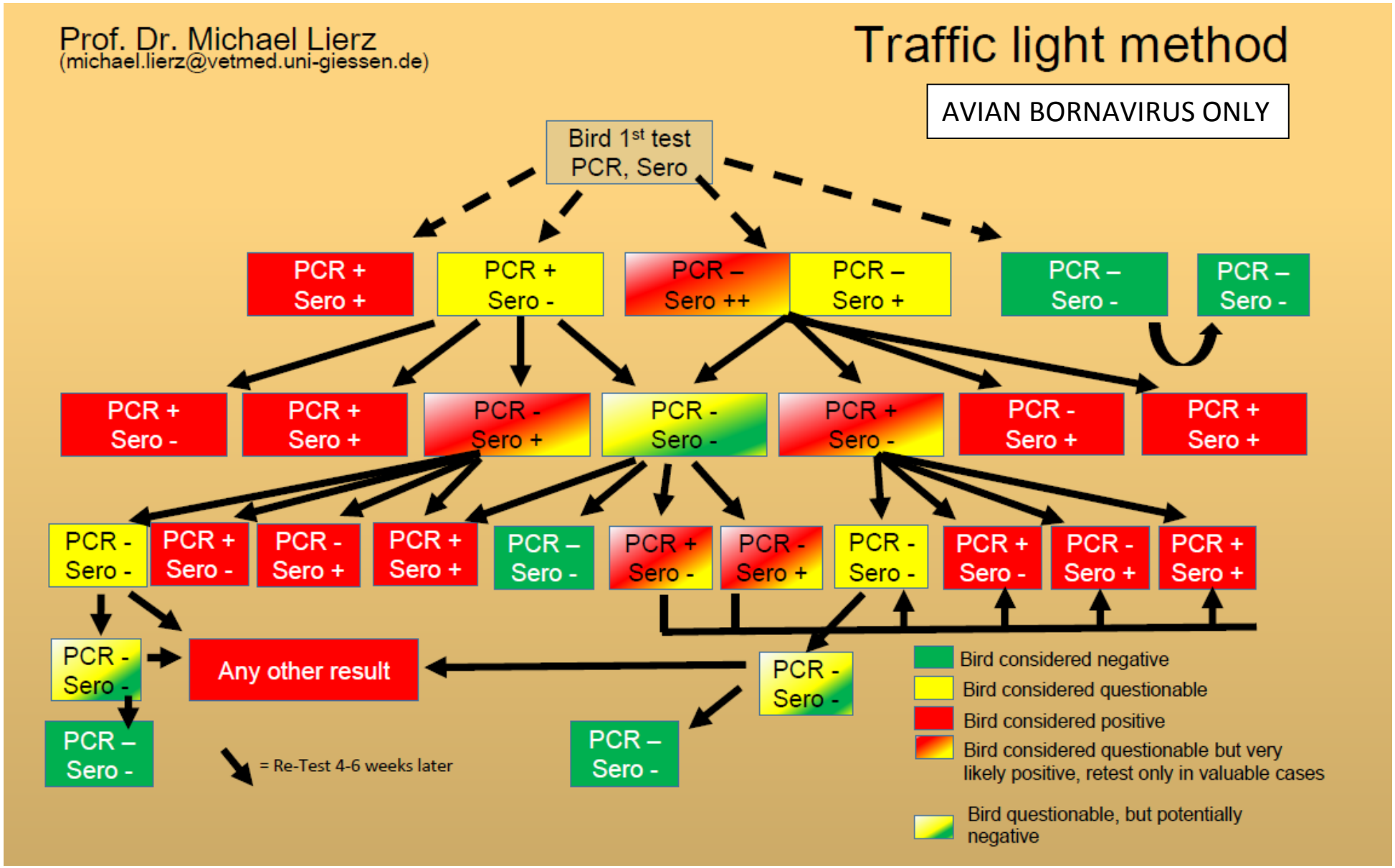
Also:
Traffic light system- see Appendix 1 for testing and clearing a flock from Avian Bornavirus

Appendix I – Avian Bornavirus flock management (only to be followed for Avian Bornavirus)



Appendix I – Avian Bornavirus flock management (only to be followed for Avian Bornavirus)