Annex K

Guidelines for pathologists and pathology laboratories for the handling of tissues from patients with, or at risk of, CJD or vCJD

Introduction

K1. This guidance is aimed at pathologists and individuals working in pathology laboratories who handle tissues from patients. It aims to ensure that laboratory staff are aware of risk factors for Creutzfeldt-Jakob disease (CJD) or variant CJD (vCJD) prior to carrying out procedures on tissues.

For the purposes of this guidance document, sporadic CJD, iatrogenic (accidentally transmitted) TSE and genetic TSE will all be referred to under the umbrella name of 'CJD'. Variant CJD will be referred to as 'vCJD'.

K2. The following information is outlined in this Annex:

- How to identify a potential case of CJD or vCJD prior to handling tissues from a living patient, or performing an autopsy on a patient with a history of dementia or a progressive neurodegenerative disorder
- the procedures for handling tissues of high or medium levels of infectivity from patients with, or at risk from, CJD or vCJD
- what to do in the event that a routinely handled tissue sample is subsequently found to be from a patient with, or at risk of, CJD or vCJD

K3. Other relevant information already covered by the ACDP TSE Working Group guidance includes:

- general laboratory containment and control measures. These are covered by Part 3 of this guidance
- the handling of brain biopsy tissues from patients with progressive neurological disorders. This is covered by Annex I of this guidance
- the procedures needed for an autopsy on a patient with, or at risk of, all forms of CJD. This is covered by Annex H of this guidance

K4. An algorithm is included to enable easy decision making on the treatment of tissue samples from patients with or at risk of CJD or vCJD, based on patient diagnosis and tissue infectivity risk. If the tissue sample is not designated

high or medium risk as outlined in Tables K1 and K2, no special precautions are needed, and normal protocols may be followed.

Patient Classification

K5. Patients are classified as follows:

A) Symptomatic

Symptomatic patients are classified according to verified WHO clinical and pathological criteria for:

a. Sporadic CJD
b. Iatrogenic (accidentally transmitted) TSE
c. Genetic TSE (familial CJD, GSS and FFI), and;
d. variant CJD (vCJD)

More information is available in Annex B of this guidance.

B) Asymptomatic patients at risk of familial forms of CJD

A patient should be considered to be at risk from familial forms of CJD linked to genetic mutations if they have or have had:

a. Genetic testing, which has indicated that they are at significant risk of developing CJD or other prion disease
b. A blood relative known to have a genetic mutation indicative of familial CJD
c. 2 or more blood relatives affected by CJD or other prion disease

C) Asymptomatic patients at risk of CJD or vCJD from iatrogenic exposure

Risk	Patient at risk of CJD	Patient at risk of vCJD
Received hormone derived from human pituitary glands, e.g. growth hormone or gonadotrophin. In the UK, the use of human-derived growth hormone was discontinued in 1985, and the use of human-derived gonadotrophin was discontinued in 1973, but human-derived products may have continued to be used in other countries	Yes	No
Undergone intradural neurosurgical procedures or operations on the spinal cord prior to August 1992	Yes	No
Received blood, tissues or organs from a donor who went on to develop CJD or vCJD, or was at risk of CJD or vCJD	Yes	Yes

Risk	Patient at risk of CJD	Patient at risk of vCJD
Received blood components or plasma derivatives from implicated batches of blood (**this includes most adult haemophiliacs in the UK**)	No	Yes
Donated blood to an individual who was later found to have vCJD	No	Yes

K6. **Autopsy on patients with dementias, ataxia and other undiagnosed neurological disorders**

In patients with a history of a neurodegenerative disorder, including dementia and ataxia, where the diagnosis is uncertain, the possibility of CJD should be considered before the autopsy is undertaken. Based on the clinical criteria listed above, CJD should be considered when there is a history of progressive dementia of less than 2 years duration when accompanied by myoclonus, visual problems, ataxia, pyramidal or extrapyramidal features, or akinetic mutism. The results of EEG studies should be examined to see if changes suggestive of CJD were present, and a positive assay for 14-3-3 protein in the CSF may also support the diagnosis of CJD.

The National CJD Surveillance Unit can provide advice on individual cases upon request – please call 0131 537 1980.

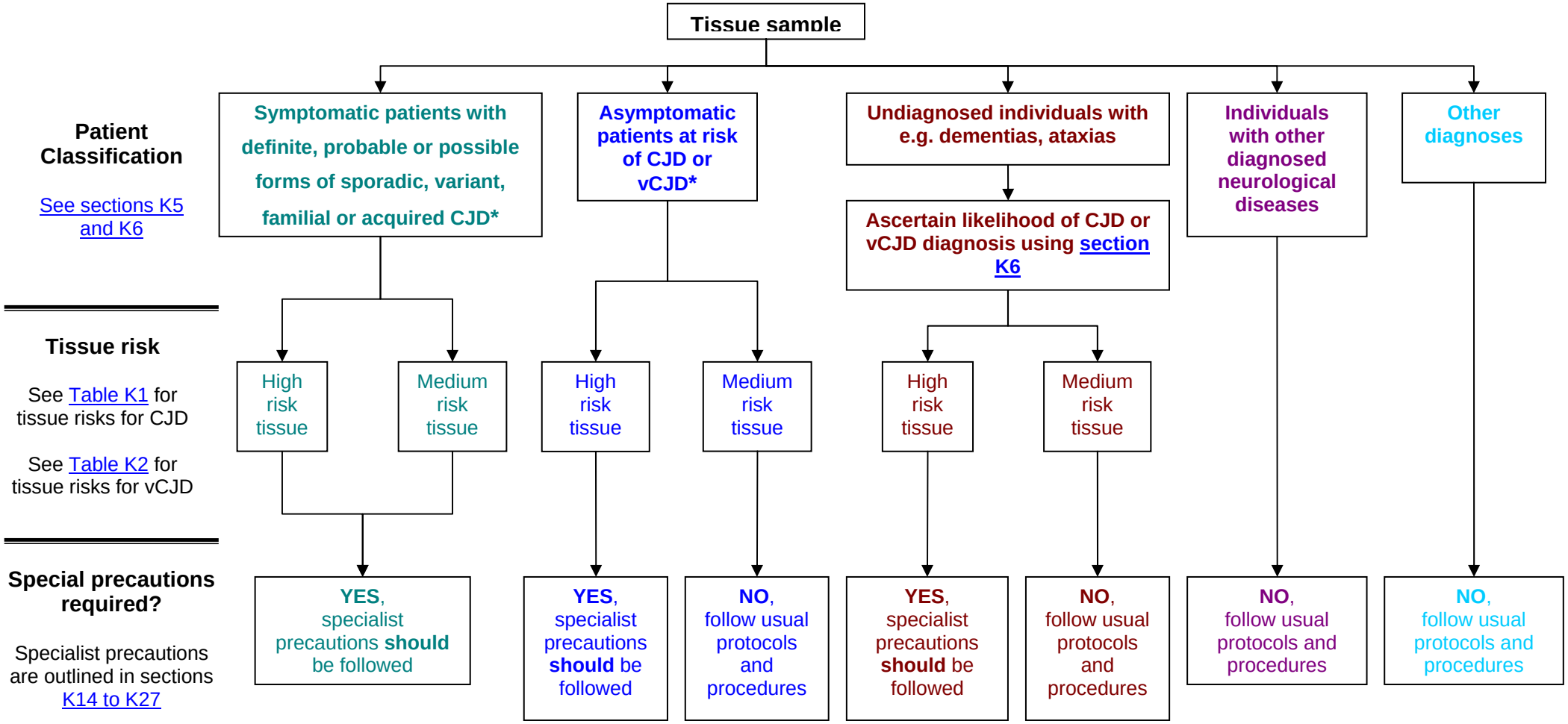
K7. **Autopsy on patients with known or suspected CJD or vCJD**

Annex H provides advice on autopsies on patients known or suspected to have CJD or vCJD.

Clinical information

K8. The clinical information on the request form accompanying the specimen should state the relevant information, and/or there may be such information on the hospital electronic patient record systems. If the patient is symptomatic, or asymptomatic but 'at risk', then the appropriate staff **must** be informed **prior** to any procedures being carried out. The samples should be handled only by fully trained staff who are aware of the relevant laboratory handling and health and safety guidelines.

Algorithm for the processing of tissue from patients with or at risk of CJD including vCJD



* See section K5

Table of tissues assumed to have medium or high level infectivity for CJD other than vCJD

K9. Table K1 outlines those tissues either assumed or proven to have medium or high level infectivity for CJD other than vCJD. This table is based on Table A1 in Annex A1 of this guidance, which is updated regularly in accordance with international guidelines. **Tissues assumed or proven to have either low level, or no, infectivity have not been included in this table, e.g. liver, kidney, muscle.**

Table K1 – tissues either assumed or proven to have medium or high level infectivity for CJD other than vCJD

Key: **+ve** = tested positive

-ve = tested negative

P = infectivity proven in experimental transmission studies

PrP^{TSE} = disease associated form of the prion protein

Tissue	Presence of abnormal prion protein and level of infectivity for CJD other than vCJD	
	PrP^{TSE} detected	**Assumed level of Infectivity**
Brain	+ve	High **P**
Spinal cord	+ve	High **P**
Dura mater*	-ve	High
Cranial nerves, specifically: o the entire optic nerve o only the intracranial components of the other cranial nerves	+ve	High
Cranial nerve ganglia	+ve	High
Posterior eye	+ve	High **P**
Pituitary gland	+ve	High
Spinal ganglia	+ve	Medium
Anterior eye and cornea**	-ve	Medium
Olfactory epithelium	+ve	Medium

* *Dura mater* is considered a high risk tissue, despite no PrP^{TSE} being detected. It is likely that *dura mater* used for grafting, and subsequently implicated in CJD transmission, could have been contaminated with brain tissue

** Anterior eye and cornea are considered medium risk, despite negative PrP^{TSE} tests, because corneal transplantation has been associated with CJD transmission

Notable omissions

K10. Peripheral tissues have been found to carry low levels of prion protein and/or infectivity in sporadic CJD. They are considered low risk due to a lack of epidemiological evidence of transmission risk.

K11. CSF and blood are classified as low risk tissues and do not require special precautions for biochemical or cytological investigations.

Table of tissues assumed to have medium or high level infectivity for vCJD only

K12. Table K2 outlines those tissues either assumed or proven to have medium or high level infectivity for vCJD. This table is based on Table A1 in Annex A1 of this guidance, which is updated regularly in accordance with international guidelines. **Tissues assumed or proven to have either low level, or no, infectivity have not been included in this table, e.g. liver, kidney, muscle.**

Table K2 – tissues either assumed or proven to have medium or high level infectivity for vCJD only

Key: **+ve** = tested positive

-ve = tested negative

NT = not tested

P = infectivity proven in experimental transmission studies

PrP^{TSE} = disease associated form of the prion protein

Tissue	**Presence of abnormal prion protein and level of infectivity for vCJD**	
	PrP^{TSE} detected	**Assumed level of infectivity**
Brain	+ve	High **P**
Spinal cord	+ve	High **P**
Dura mater*	-ve	High

Cranial nerves, specifically: o the entire optic nerve o only the intracranial components of the other cranial nerves	+ve	High
Cranial nerve ganglia	+ve	High **P**
Posterior eye	+ve	High
Pituitary gland	+ve	High
Spinal ganglia	+ve	Medium **P**
Anterior eye and cornea**	-ve	Medium
Olfactory epithelium***	NT	Medium
Tonsil	+ve	Medium **P**
Appendix	+ve	Medium
Spleen	+ve	Medium **P**
Thymus	+ve	Medium
Other lymphoid tissues (such as gut-associated lymphoid tissues and lymph nodes)	+ve	Medium **P**

* *Dura mater* is considered a high risk tissue, despite no PrP^{TSE} being detected. It is likely that *dura mater* used for grafting, and subsequently implicated in CJD transmission, could have been contaminated with brain tissue.

** Anterior eye and cornea are considered medium risk, despite negative PrP^{TSE} tests, because corneal transplantation has been associated with CJD transmission

*** Olfactory epithelium is considered medium risk, despite not having been tested for the presence of PrP^{TSE}, because PrP^{TSE} is present in the olfactory tract and bulb in CJD

Notable omissions

K13. Blood is considered to have a low risk of infectivity despite the four cases of vCJD infection that have been transmitted by contaminated blood transfusions. It is thought that the large volume and multiple infectious doses are important factors in transmission by packed red cells and other blood components. Blood is therefore classified as a low risk tissue and does not require special precautions for biochemical or cytological investigations

K14. CSF is classified as a low risk tissue and does not require special precautions for biochemical or cytological investigations.

Extra precautions for handling tissues with high or medium infectivity from patients with, or at risk of, all forms of CJD and vCJD

Trimming tissue

K15. Wearing the appropriate protective personnel equipment (PPE), **all fresh tissue** should be sliced or trimmed in a Class 1 Microbiological safety cabinet using cut resistant/steel mesh gloves and, when possible, disposable instruments. A disposable paper lining can be placed over the base of the cabinet to help contain splashes of contaminated formalin. After trimming, the cabinet should be wiped down with 2M sodium hydroxide and left for 1 hour. Do not use 20,000pmm sodium hypochlorite in cabinets as this will corrode the metal. Disposable instruments and sharps can be chemically decontaminated with 2M sodium hydroxide for 1 hour prior to placing them in a suitable container for disposal. Waste contaminated formalin should be absorbed into sawdust in a combustible stout container with lid prior to disposal. All this waste, including gowns, gloves and aprons should be sent for incineration. Non-disposable instruments and cut resistant/steel mesh gloves should be autoclaved or decontaminated with 2M sodium hydroxide for 1 hour.

K16. **Fixed tissues** can be trimmed either in a Class 1 Microbiological Safety cabinet or on a ventilated bench, taking care not to allow contamination of the surrounding laboratory space. Cleaning and disposal can be performed as above.

Treatment with formic acid prior to processing – to reduce infectivity

K17. Treatment with formalin appears to improve the action of the formic acid in reducing infectivity of the sample. The efficacy of formic acid in reducing infectivity in tissue samples from patients with CJD has been proven in the literature[1].

K18. Using a cabinet/fume cabinet, depending on local risk assessment, fixed tissue samples should be immersed in 96% formic acid for 1 hour. Samples must be thoroughly washed with water prior to further processing and examination.

[1] Brown P, Wolff A, Gajdusek DC. A simple and effective method for inactivating virus infectivity in formalin-fixed tissue samples from patients with Creutzfeldt-Jakob disease. Neurology. 1990; 40:887-90

K19. Formic acid is used normally in pathology laboratories to decalcify bones, and must be used in a cabinet/fume cupboard. It does not appear to harm the morphology of the tissues, so the results of subsequent tests are not jeopardised. In addition, plastic cassettes and other equipment are not affected. Tissues treated with formic acid can become slightly brittle, but this is not usually a major problem at the time of microtomy.

K20. **Following treatment with formic acid, tissues should be processed and handled like any other routine pathological specimen.** The processing fluids used on tissue blocks that have been exposed to formic acid (including high infectivity tissues e.g. brain) should not be treated as potentially contaminated and can be disposed of using routine precautions.

Microtomy

K21. Sections can be cut on a routine microtome using disposable blades. The blades should be discarded prior to cutting a different case. Disposable microtome blades are available, and are used routinely by many laboratories.

Frozen section work

K22. It is advised that **no** frozen section work should be done on **high risk tissues** for patients with, or at risk of, CJD or vCJD.

Disinfection and decontamination

K23. **Factors to consider when choosing chemical deactivation**

a. Sodium Hypochlorite

- Requires a dilution yielding 20,000 ppm available chlorine
- Must not be used on open surfaces i.e. benches due to harmful fumes
- Corrodes metal and steel - do not use on cabinets (max. only 5,000 ppm)
- Incompatible with formaldehyde, alcohols and acids – explosive
- Can be used for disinfecting glassware in a container within a cabinet
- Concentrated stock dilutions last for only approximately 2-3 weeks
- Diluted solutions are very unstable and should be made up daily

b. Sodium Hydroxide

- Use as 2M sodium hydroxide
- Should not be used on aluminium or zinc
- Will not cause fumes but corrosive to body tissue
- Irritant and harmful as dust

Table K3 – Decontamination and disposal

Item	Action	Contact Time
Formaldehyde-fixed tissues	Immersion in 96% formic acid (**unless** tissue has previously been exposed to phenol, which interacts deleteriously with formic acid)	60 minutes
Disposable clothing, paper tissues, etc	Double bag and dispose of by incineration	
Disposable sharps and instruments	Decontaminate with 2M sodium hydroxide for 1 hour. Collect in a suitable container, double bag and dispose of by incineration	
Non-disposable metal instruments	Soak in 2M sodium hydroxide, then autoclave at 134^{O}C with holding time of 3 minutes	60 minutes
Glassware including microscope slides	Collect in a suitable container, double bag and dispose of by incineration	60 minutes
Work surfaces / Microtome for non formic treated blocks	Flood with 2M sodium hydroxide, then wash well	60 minutes
Microbiological Safety Cabinets	Wash liberally with 2M sodium hydroxide, then wash well. Double bag filters and dispose of by incineration	60 minutes
Xylene, chloroform, contaminated water, contaminated fluids e.g. formalin, solvents *	Absorb on sawdust in a combustible stout container (with lid), double bag and then dispose of by incineration	
Paraffin wax and wax shavings	If tissues have been treated with formic acid, handle like any other routine pathological specimen. If not, collect in stout container (with lid) then dispose of by incineration	
CSF, Blood	Absorb on sawdust in a stout combustible container (with lid) then dispose of by incineration	60 minutes

* **For all liquid disposals, be careful to avoid dangerous chemical or explosive incompatibilities before disposal**

Decontamination of specialist equipment

K24. Providing other specialist pathology equipment is used after formic acid treatment, no decontamination of equipment is required.

Retrospective diagnosis of CJD following normal tissue sample processing

K25. The situation may arise whereby a patient is not suspected of having CJD, but is subsequently diagnosed following a biopsy or autopsy.

K26. The following needs to be taken into account when considering what action to take:

- The type of tissue involved
- The type of CJD that has been diagnosed
- How recently the diagnosis was made following the laboratory processes carried out on this tissue
- Whether the patient had clinical symptoms of CJD, or was in one of the "at risk" groups
- Whether the case has been notified to the National CJD Surveillance Unit (see section K28) and whether any of the tissue should be referred for further specialist investigations

K27. The following actions should be undertaken **(please note that these actions do not constitute best practice but reflect the best course of action to be taken under these particular circumstances)**:

a. Any samples taken from the patient should be traced, to ascertain what the tissue had been used for. Establish if any residual tissue was being stored
b. Fixed tissue should be double bagged and sent for incineration after the statutory 6 weeks. Prior to incineration, the NCJDSU should be contacted to ascertain whether they have a use for the tissue (see section K28)
c. Frozen tissue, including cryostat sections, should be fixed and then double bagged and sent for incineration after the statutory 6 weeks. Prior to incineration, the NCJDSU should be contacted to ascertain whether they have a use for the tissue (see section K28)
d. The paraffin blocks should be removed from main store and filed separately, clearly labelled as form of CJD and then stored separately. Reprocessing the blocks using formic acid is not required; the blocks can be sent for further investigations in the National CJD Surveillance Unit (see section K28)

e. Mounted (cover-slipped) slides can be filed as per usual

f. Un-mounted slides should be sent for incineration.

g. If it is still in use, the blade of the microtome used to cut the sample should be placed in a sharps box and this should be disposed of by incineration. The water in the water bath should be changed

h. **For brain banking**, identify the brass plates used for dissection of the CJD sample. They can no longer be used for the dissection or freezing of brain tissues. Prions are known to bind to metal surfaces, and washing with water will not guarantee the removal of prion infectivity. Frozen tissue from the next two cases received and cut fresh on the same brass plate following the CJD sample should be identified and withdrawn from research and either stored with appropriate biohazard labelling or transferred to the National CJD Surveillance Unit (see section K28).

   It is recommended that brass plates used on unfixed tissues in brain banks should be washed and soaked in sodium hypochlorite between dissections of new cases.

Where to send tissue samples for CJD testing

K28. If any tissue sample requires further investigations for the possibility of CJD infection, please contact Professor James Ironside or Mrs Linda McCardle in the National CJD Surveillance Unit on 0131 537 1980.

If any type of CJD has been diagnosed in an individual, please notify the National CJD Surveillance Unit on 0131 537 1980