

## 9. APPENDICES

### Appendix 1 – Summary of commercially available anti-cTnI antibodies that have reactivity against bovine and/or porcine cTnI

Clone (monoclonal only)	Immunogen	Epitope	Isotype	Tested applications	Tested species reactivities	Supplier(s)	Comments / actions
[10B11]	FL hu	16-20	ms IgG1	WB, ELISA, IP	hu, po, cn, rb, fe, rt, (-bv)	HyTest	Reacts with porcine but not bovine cTnI
[4C2]	FL hu	22-29	ms IgG2a	WB, ELISA, IP	hu, bv, po, gt, cn, rb, fe, rt, ms	HyTest	Used during project Q01105, also reacts with sh cTnI
[19C7]	FL hu	41-49	ms IgG2b	WB, ELISA	hu, bv, po, gt, cn, rb, fe, rt, ms, fs	HyTest	
[414]	FL hu	55-61	ms IgG1	WB, ELISA, IP	hu, bv, po, cn, rb, ms, fs	HyTest	
[8E10]	FL hu	86-90	ms IgG1	WB, ELISA	hu, bv, po, gt, cn, rb, fe	HyTest	
[16A11]	FL hu	86-90	ms IgG1	WB, ELISA	hu, bv, po, gt, cn, rb, fe	HyTest	
[MF4]	FL hu	190-196	ms IgG1	WB, ELISA	hu, bv, po, gt, cn, fe, rt, ms	HyTest	
[22B11]	FL hu	20-24 non-phospho	ms IgG2b	WB, ELISA	hu, po, (-bv)	HyTest	Reacts with porcine but not bovine cTnI.
[247]	FL hu	65-74	ms IgG	WB, ELISA	hu, bv, po, gt, cn, rb, fe, rt, ms	HyTest	
[10F4]	FL hu	34-37	ms IgG2a	WB, ELISA	hu, rt, gt, fe, rb, bv, cn, po	HyTest	
	hu 1-100	1-100	polyclonal rb IgG	WB, IHC	hu, ms, po predict: rt	Abcam ab47003	May provide speciation, although not tested by ELISA.

	hu 8-35	8-35	polyclonal ck IgY	WB	hu, bv, ms, rb predict: fe, rt	Abcam ab31213, Acris & Novus NBP1-05004, LS-C96080	May provide speciation, although not tested by ELISA.
[B2]	FL cow		ms IgG2b	WB, ELISA, IP	hu, bv, rb, ms	AbD, Acris, SC, Thermo	May provide speciation?
[110]	FL hu	34-37	ms IgG2a	WB, ELISA	hu, bv, po, gt, rb, ca, fe, ms, rt	Acris	
[581]	FL hu	143-152	ms IgG	WB, IHC, IP	hu, bv, po, ca, rt, ms	Acris	
[2Q1100]	FL cow	87-93	ms IgG2b	WB, ELISA, IHC	hu, bv, rb, ms, ch, fr	Acris, Novus	Also reacts with skel. muscle Tnl
	FL hu	55-95	rb polyclonal IgG	WB, ELISA, IP, IF	hu, bv, po, gt, cn, rb, fe, rt, ms	SC-98830	
[1.BB.141]	FL hu	16-20	ms IgG1	WB, IP	hu, po, cn, fe, rt	SC	May provide speciation, although not tested by ELISA.
[1.BB.145]	FL hu	41-49	ms IgG2b	WB, IP	hu, bv, po, cn, fe, rt, ms	SC	
[2Q1108]	Hu Tn complex	41-49	ms IgG2b	WB, IP	hu, bv, po, cn, fe, rt, ms	SC	

*Abbreviations: FL – full length; Species abbreviations: hu – human, bv – bovine, po – porcine, gt – goat, cn – canine, rb – rabbit, fe – cat, rt – rat, ms – mouse, fs – fish, hs – horse, pm – primate, ck – chicken, gp – guinea pig, sh, sheep, tk – turkey, fr – frog. Applications abbreviations: ELISA – Enzyme-linked Immunosorbent assay, WB – Western Blotting, IHC – Immunohistochemistry, IP – Immunoprecipitation, IF – Immunofluorescence.*

**Appendix 2 – Summary of commercially available anti-cTnT antibodies that have reactivity against bovine and/or porcine cTnT**

Clone (monoclonal only)	Immunogen	Epitope	Isotype	Tested applications	Tested species reactivity	Supplier(s)	Comments / actions
[7F4]	FL hu	60-70	ms IgG2b	WB, ELISA	hu, po	HyTest	May provide speciation?
[1F2]	FL hu	60-71	ms IgG1	WB, ELISA	hu, bv, gt, cn, rb, fe, fs, (-po)	HyTest	Reacts with bovine but not porcine cTnT.
[7G7]	FL hu	60-71	ms IgG1	WB, ELISA	hu, bv, (-po)	HyTest	Reacts with bovine but not porcine cTnT.
[3D6]	FL hu	71-95	ms IgG1	WB, ELISA	hu, bv, gt, cn, rb, ms, fs, (-po)	HyTest	Reacts with bovine but not porcine cTnT.
[2F3]	FL hu	95-181	ms IgG2b	WB, ELISA	hu, bv, po, gt, cn, rb, fe, rt, ms, fs	HyTest	
[1A11]	FL hu	95-181	ms IgG2b	WB, ELISA	hu, bv, po, gt, cn, rb, fe, rt, ms, fs	HyTest	
[2G3]	FL hu	95-181	ms IgG2b	WB, ELISA	hu, bv, po, gt, cn, rb, fe, rt, ms	HyTest	
[1F11]	FL hu	146-160	ms IgG2b	WB, ELISA	hu, bv, po, gt, cn, rb, fe, rt, ms, fs	HyTest	Used during project Q01105, also reacts with sh cTnI
[7A9]	FL hu	180-287	ms IgG1	WB, ELISA	hu, bv, po, gt, cn, rb	HyTest	
[0.N.590]	rb peptide FMPNLVPPKI	FMPNLVPPKI	ms IgG1	WB, IHC	hu, po, cn, rb, rt, ms, fs, ck, gp predict: fe, sh, tk	Abcam	
[5C12]	FL cow	180-258	ms IgM	WB, ELISA, IP	bv	Abcam, Acris, GeneTex, GenWay, LS Bio C89791, Novus, SC, Thermo,	May provide speciation? IgM potentially less stable than IgG

[TT44] (BGN/1288/44)		FMPNLVP PKI 70-80	ms IgG1	WB, ELISA	broad	AbD, Acris, GenWay, Novus, SC, Thermo	
[TT47] (BGN/1288/47)		FMPNLVP PKI 70-80	ms IgG1	WB, ELISA	broad	AbD, Acris, GenWay, Novus, SC, Thermo	
[BGN/1288/2F 8]		FMPNLVP PKI 70-80	ms IgG1	IHC	broad	AbD, LS Bio (LB- B3651)	
[13-11]	FL rb	FMPNLVP PKI 70-80	ms IgG1	IF, IP	broad	Acris, LS Bio (LS- C88032)	
no clone given	FL cow		ms IgG1	WB, ELISA, IP	hu, bv, gt, fe, cn, fs, rb, (-po)	LS Bio C89787	Reacts with bovine but not porcine cTnT.
no clone given	FL cow	60-70	ms IgG1	WB, ELISA, IP	hu, bv, fe, cn, fs, gt, rb, (-po)	LS Bio C26391	Reacts with bovine but not porcine cTnT.
no clone given	FL cow		ms IgG1	WB, ELISA, IP	hu, bv	LS Bio C42199	May provide speciation?
	FL hu		msIgG2b	WB, ELISA, IP	hu, bv, po, fe, cn, fs, gt, rb, rt, ms	LS Bio C89788	
[1.BB.155]	FL cow	60-70	ms IgG1	WB, IP	hu, bv, cn, fe	SC	May provide speciation, although not tested by ELISA.
[1.BB.157]	FL hu	60-70	ms IgG1	WB, IP	hu, bv	SC	May provide speciation, although not tested by ELISA.
[5E842]	FL cow	180-258	ms IgM	WB	bv	SC	May provide speciation, although not tested by ELISA.
[5E845]	FL cow	60-70	ms IgG1	WB, IP	hu, bv, cn, fe	SC	May provide speciation, although not tested by ELISA.
[CT3]	FL cow		ms IgG2a	WB, IP, IF	broad	SC	

	FL hu	C-terminus	gt polyclonal IgG	WB, ELISA, IP, IF	hu, bv, cn, rt, ms	SC-8121	May provide speciation?
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*Abbreviations: FL – full length; Species abbreviations: hu – human, bv – bovine, po – porcine, gt – goat, cn – canine, rb – rabbit, fe – cat, rt – rat, ms – mouse, fs – fish, hs – horse, pm – primate, ck – chicken, gp – guinea pig, sh, sheep, tk – turkey, fr – frog. Applications abbreviations: ELISA – Enzyme-linked Immunosorbent assay, WB – Western Blotting, IHC – Immunohistochemistry, IP – Immunoprecipitation, IF – Immunofluorescence.*

### **Appendix 3 - Summary of commercially available anti-SP-A antibodies**

Clone (monoclonal only)	Immunogen	Epitope	Isotype	Tested applications	Tested species reactivities	Supplier(s)	Comments / actions
[6F10]	FL hu		ms IgG1	WB, ELISA, IHC, IF	hu	Abcam, Acris, Enzo, Novus, SC, Thermo	
[32E12]	hu 104-246	104-246	ms IgG2a	IHC	hu	Abcam, Abnova	
	hu 221-236	221-236	polyclonal rb IgG	WB	hu, rt predict: cn, po	Abcam ab103789	May react with porcine SP-A, although not tested by ELISA
	hu		polyclonal rb IgG	WB	hu predict: po, pm	Abcam ab87674	May react with porcine SP-A, although not tested by ELISA
	FL hu		polyclonal gt IgG	WB, ELISA	hu	Abcam ab78462	
	FL bv		polyclonal rb	WB, ELISA	bv, sh	Abcam ab78173, LS Bio C17951, Millipore AB3424	Used during project Q01105
[1G8]	FL hu		ms IgG1	WB, ELISA	hu	Abnova	
[3E3]	FL hu		ms IgG1	WB, ELISA	hu	Abnova	
[3H2]	FL hu		ms IgG1	WB, ELISA	hu	Abnova	
[4C4]	FL hu		ms IgG1	WB, ELISA	hu	Abnova	
[4D6]	FL hu		ms IgG1	WB, ELISA	hu	Abnova	
	hu	221-236	polyclonal rb IgG	WB	hu, rt	Acris BB-PA1347	
	FL hu		polyclonal rb IgG	WB, IP, IHC	hu	Acris 11850-1-AP	
[MAB0227]	FL hu		ms IgG1	WB, ELISA, IHC	hu	Acris	

	FL hu		rb polyclonal	WB, ELISA	hu, ms, pm	GenWay 18-251-404571	
[PE10]	FL hu	C-terminal	ms IgG2b	IHC	hu, po*	Immuno-Biological Laboratories	Reacts with porcine SP-A, although not tested by ELISA
	FL hu		polyclonal rb	WB, ELISA, IHC	hu, rt, ms, pm	LS Bio C17949, Millipore AB3420	
	FL hu		polyclonal gt	WB, ELISA	hu	LS Bio C17950, Millipore AB3422	
[3-4]	FL cn		ms IgG	ELISA	cn	LS Bio , Millipore	
[H3]	FL hu		ms IgM	WB, ELISA	hu	LS Bio C17952, Millipore	
[1-6]	FL cn		ms Ig	ELISA	cn	LS Bio , Millipore	
[#10]	FL hu		ms IgG3	WB, ELISA	hu	LS Bio C17955, Millipore	
	hu 101-248	101-248	polyclonal rb IgG	WB, ELISA, IP, IF, IHC	hu, rt, ms	SC-13977	
	hu	N-term	polyclonal gt IgG	WB, ELISA, IP, IF	hu, po, rt, ms, hs	SC-7700	May cross-react with LOC387911 and CTRP9. Reacts with porcine SP-A
	hu	C-term	polyclonal gt IgG	WB, ELISA, IP, IF	hu, po, rt, ms, hs	SC-7699	Reacts with porcine SP-A
[HYB238]	FL hu		ms IgG1	WB, ELISA, IHC	hu	SC, Thermo	

*Abbreviations: FL – full length; Species abbreviations: hu – human, bv – bovine, po – porcine, gt – goat, cn – canine, rb – rabbit, fe – cat, rt – rat, ms – mouse, fs – fish, hs – horse, pm – primate, ck – chicken, gp – guinea pig, sh, sheep, tk – turkey, fr – frog. Applications abbreviations: ELISA – Enzyme-linked Immunosorbent assay, WB – Western Blotting, IHC – Immunohistochemistry, IP – Immunoprecipitation, IF – Immunofluorescence.*

\* The epitope of SP-A recognised by [PE10] has been mapped to the C-terminus of human SP-A (Hiraike et al., 1995) and the antibody has been used to detect porcine SP-A by immunohistochemistry (Paananen et al., 1999).

#### **Appendix 4 - Summary of commercially available anti-PRDX6 antibodies**

Clone (monoclonal only)	Immunogen	Epitope	Isotype	Tested applications	Tested species reactivities	Supplier(s)	Comments / actions
	FL rt		polyclonal rb IgG	WB, ELISA, IHC	hu, rt, ms	Abcam ab59543	Used during project Q01105, reacts with bv, po & sh PRDX6.
	FL hu		polyclonal rb IgG	WB, IHC	hu, rt, ms	Abcam ab16824	
	hu peptide		polyclonal rb IgG	WB, IHC	hu, rt, ms predict: bv, po, ck, pm	Abcam ab73350	
	hu peptide		polyclonal rb IgG	WB, IHC	hu, rt, ms predict: bv, po, ck, pm	Abcam ab73683	
	FL rt		polyclonal sh IgG	WB, ELISA, IHC	hu, rt, ms	Abcam ab59544	
	hu peptide		polyclonal rb IgG	WB	hu	Abcam ab28444	
[8H11]	FL hu		ms IgM	WB, IHC	hu, rt	Abcam, Millipore	
[1A11]	hu		ms IgG1	WB, ELISA, IP	hu	Abcam, Abnova, Acris, GenWay, Novus	
[4A3]	hu		ms IgG2b	WB, ELISA	hu, rt, ms	Abcam, Abnova, Acris, GenWay, Novus	
[5E1]	hu		ms IgG1	ELISA, IHC	hu	Abcam, Abnova, Acris, GenWay, Novus	



[ERP3755]	hu peptide		rb IgG	WB, IHC, IP	hu, rt, ms	Abcam, Acris, GeneTex, GenWay, Novus,	
[ERP3754]	hu peptide		rb IgG	WB, IHC	hu, rt, ms	Acris, GeneTex, GenWay, Novus	
[6H5]	hu		ms IgG1	ELISA, IP	hu	Abnova, Acris, GenWay, Novus	
[3A10-2A11]	FL hu		ms IgG1	WB, ELISA	hu	Abnova, Novus, Sigma	
	FL rt		polyclonal sh	WB, ELISA, IHC	hu, rt, ms	Abnova PAB8033, Acris, Novus S-073-100	
	FL hu		polyclonal rb	WB, IHC, IP	hu	Abnova PAB1191, Acris	
	hu peptide		polyclonal rb	WB	hu	Abnova PAB1305, Acris	
	C-term hu		polyclonal rb	WB, ELISA	hu	Acris, Novus AP17665PU-N	
	rt		polyclonal rb	WB, ELISA, IHC	hu, rt, ms	Acris, Novus R-169-100	
	hu		polyclonal rb	WB, IHC	hu, ms predict: bv, rt	GeneTex GTX115262	
	hu		polyclonal rb	WB, IP	hu, rt, ms	GenWay 18-007-283010	
	FL rt		polyclonal rb	WB, IHC	hu, rt	Millipore AB9246	
	hu peptide		polyclonal rb IgG	WB, ELISA	hu predict: bv, po, ms, pm	Novus NBP1-49402	
	196-210 hu	196-210	polyclonal rb	WB, IP	hu, rt, ms	Sigma P0058	immunogen shares 100% identity with bv & po sequence
	11-27 hu	11-27	polyclonal rb	WB, IP	hu, rt, ms	Sigma P0050	immunogen shares 96% identity with bv & po sequence

	175-224 hu	175-224	polyclonal rb	WB	hu	Sigma AV48268	immunogen shares 98% identity with bv & po sequence
	144-192	144-192	polyclonal rb IgG	WB	hu	Sigma AV48267	immunogen shares 96% identity with bv & po sequence
	3-150 hu		polyclonal rb	WB, IHC, IF	hu	Sigma HPA 006983	
	rt N-terminal peptide	N-terminus	polyclonal gt IgG	WB, ELISA, IP, IF	hu, bv, po, rt	SC-55015	
[D9]	hu	80-110	ms IgG1	WB, ELISA, IP, IF	hu	SC	
	hu peptide (internal)	internal	polyclonal gt IgG	ELISA	hu, bv, po	SC-55017	
	hu peptide (internal)	internal	polyclonal gt IgG	WB, ELISA, IP, IF	hu, bv	SC-55013	
[36]	FL hu		ms IgG1	WB, ELISA	hu	SC	
[52]	FL hu		ms IgG1	WB, ELISA, IP	hu	SC	
[F12]	FL hu		ms IgG1	WB, ELISA, IP, IF	hu, rt, ms	SC	
	FL hu		polyclonal rb IgG	WB, ELISA, IP, IF	hu, bv, po, cn,, rt, ms, hs	SC-134478	
	rt		polyclonal rb	WB, ELISA, IHC	hu, rt, ms	Thermo PA1-18277	
	rt		polyclonal sh	WB, ELISA, IHC	hu, rt, ms	Thermo PA1-18281	

*Abbreviations: FL – full length; Species abbreviations: hu – human, bv – bovine, po – porcine, gt – goat, cn – canine, rb – rabbit, fe – cat, rt – rat, ms – mouse, fs – fish, hs – horse, pm – primate, ck – chicken, gp – guinea pig, sh, sheep, tk – turkey, fr – frog. Applications abbreviations: ELISA – Enzyme-linked Immunosorbent assay, WB – Western Blotting, IHC – Immunohistochemistry, IP – Immunoprecipitation, IF – Immunofluorescence.*

## **Appendix 5 – Questionnaire circulated to PA laboratories by Defra**

### **Review of facilities in Public Analyst Laboratories**

At Nottingham Trent University we have been commissioned to develop semi-quantitative Enzyme Linked Immunosorbent Assays (ELISAs) for the detection of offal in meat and meat products. In order to facilitate knowledge transfer and develop appropriate methodologies we need to know what equipment you have available for cooking, centrifugation, and ELISA plate assays. We would appreciate answers to the following questions to help us plan the project for the outset.

- (1) In your laboratory what facilities are available for cooking raw meat and meat products? e.g. oven (temperature range), microwave oven, water bath (temperature range)
  
- (2) In your laboratory what facilities are available for centrifugation (including tube size/volume and maximum speed)?
  
- (3) Do you use a plate washer when performing ELISAs, if so, what make & model?
  
- (4) What make/model of plate reader do you use, including available wavelengths?
  
- (5) Do you routinely use multi-channel pipettes or repeater pipettes when performing ELISAs?