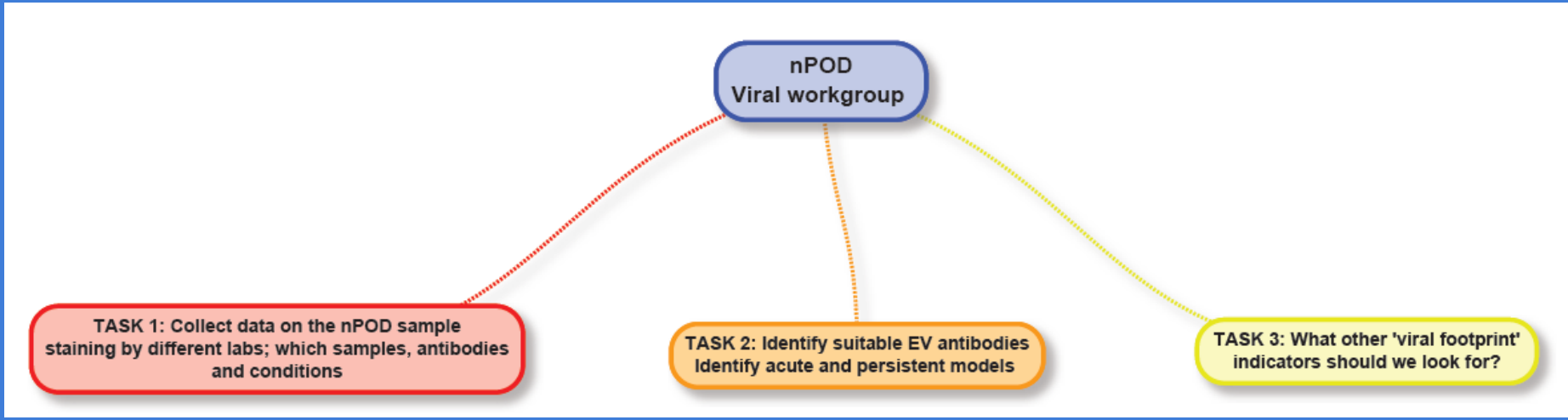
nPOD Viral Workgroup – IHC studies update



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Task 1: Collated Results

LABORATORY	OPPC ID	Disease	Location of block	FFPE/ Frozen	HIER	Primary Antibody/ Dilution	Immunohistochemistry	In Situ hybridisation
SR/NGM	14	control	Pan Body	FFPE	1mM EDTA pH8	Dako vp1 (5D8/1); 1/2000	Negative -1 possible dotta cell in 1 islet	
Tampere	14	control	PA	FFPE	Tris-EDTA pH 8	Dako vp1 (5D8/1); 1/300	na	negative
Tampere	14	control	Pan head	FFPE	Tris-EDTA pH 8	Dako vp1 (5D8/1); 1/300	na	negative
Tampere	14	control	Pan tail	FFPE	Tris-EDTA pH 8	Dako vp1 (5D8/1); 1/300	na	na
SR/NGM	6	T1D	Pan Body	FFPE	1mM EDTA pH8	Dako vp1 (5D8/1); 1/2000	6 islets containing multiple intense positive cells	
Tampere	6	T1D	PanHead	FFPE	Tris-EDTA pH 8	Dako vp1 (5D8/1); 1/300	positive	negative
SR/NGM	5	T1D	Pan Body	FFPE	1mM EDTA pH8	Dako vp1 (5D8/1); 1/2000	5 islets containing multiple intense positive cells	
Tampere	5	T1D	Pan Head	FFPE	Tris-EDTA pH 8	Dako vp1 (5D8/1); 1/300	na	na
Tampere	5	T1D	Pan Head	FFPE	Tris-EDTA pH 8	Dako vp1 (5D8/1); 1/300	negative	negative
Tampere	5	T1D	Pan Tail	FFPE	Tris-EDTA pH 8	Dako vp1 (5D8/1); 1/300	positive	na
SR/NGM	13	T1D		FFPE	1mM EDTA pH8	Dako vp1 (5D8/1); 1/2000	Numerous islets containing intense positive cells	
Tampere	13	T1D	Pan Head	FFPE	Tris-EDTA pH 8	Dako vp1 (5D8/1); 1/300	positive	negative
Tampere	13	T1D	Pan Body	FFPE	Tris-EDTA pH 8	Dako vp1 (5D8/1); 1/300	positive	negative
Tampere	13	T1D	Pan Tail	FFPE	Tris-EDTA pH 8	Dako vp1 (5D8/1); 1/300	positive	positive



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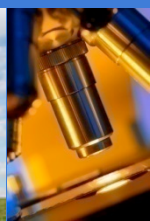

Task 1: Collated Results

- Varied IHC staining conditions (HIER, dilution, detection system, manual v automated staining)
- Good concordance so far with IHC on paraffin sections between laboratories and ISH
- This exercise has allowed us to identify those samples which should be circulated to respective labs for analysis

QUESTION: Do these laboratories test these samples using their own optimised protocols or are we going to attempt to standardise?




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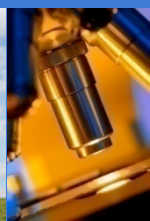

Task 2: EV antibodies

LABORATORY	Antibody Name and Host	Vendor/ Clone	FFPE/ Frozen	Optimal HIER	Primary Antibody Dilution	Secondary Antibody/ Dilution	Comments
SR/NGM	anti-enterovirus (5D8/1) mouse mAb	DAKO (M7064)	FFPE (AKF)	1mM EDTA pH8	1/500-1/2000; dependent on sample	DAKO Envision HRP	Strong staining in all infected cell culture systems, in CVB-infected mice in tissues identified by EV specific ISH, in CVB-infected neonatal heart and pancreas. The staining in the CVB-infected neonate mirrors that seen with EV specfic ISH. Some samples we do observe staining of SMC, but this is not apparent in all samples. Optimal HIER and dilution established in AKF samples, therefore may require modification in nPOD
SR/NGM	kdf anti-vp1 rabbit pAb	R Kandolf	FFPE (AKF)	1mM EDTA pH8	1/400-1/800; dependent on sample	DAKO Envision HRP	As the DAKO antibody, but backgound is generally higher. Optimal HIER established in AKF samples, therefore may require modification in nPOD
SR/NGM	Pan-enterovirus 9D5 mouse mAb	Millipore (C#3361)	FFPE (AKF)	1mM EDTA pH8	1/2; overnight incubation with primary	DAKO Envision HRP	Weak stain in infected heart, but not in pancreas
SR/NGM	Pan-enterovirus 2E11 mouse mAb	Millipore (C#3362)	FFPE (AKF)	No HIER	1/3-1/5	DAKO Envision HRP	Weak stain in infected heart, N/S stain in coxsackie-infected pancreas
SR/NGM	Pan-Enterovirus blend mouse mAbs	Millipore (C#3360)	FFPE (AKF)	10mM Tris 1mM EDTA pH9	1/3	DAKO Envision HRP	Stains infected cells, but some background in uninfected controls (see table). In tissue, this antibody is sensitive to sections drying out and autolysis. General background poor, but does produce positive stain in infected heart. In pancreas tend to see wash of background in exocrine tissue.
SR/NGM	Coxsackie B Blend	Millipore (C#3303)	FFPE (AKF)	10mM Tris 1mM EDTA pH9	1/2	DAKO Envision HRP	No convincing staining in CVB-infected cells
SR/NGM	HH anti-CVB3 rabbit pAb	Hyoty	FFPE (AKF)	10mM Tris 1mM EDTA pH9	1/200	DAKO Envision HRP	Works well in infected cell but no stain in the infected heart
SR/NGM	KK anti-CVB3 VP1 rabbit pAb	Klingel	FFPE (AKF)	10mM citrate pH6	1/500	DAKO Envision HRP	Worked well in CVB3-infected cells, however observed high background in uninfected heart and only weak positive signal in the infected.
SR/NGM	KK anti-CVB3 3D POL rabbit pAb	Klingel	FFPE (AKF)	10mM citrate pH6	1/750-1/1000	DAKO Envision HRP	Works well in CVB3-infected cells, but does not detect signal in infected heart.
SR/NGM	KK anti-CVB3 VP1 II rabbit pAb	Klingel	FFPE (AKF)	10mM citrate pH6	1/500	DAKO Envision HRP	Works well in CVB3-infected cells, but does not detect signal in infected heart.
SR/NGM	anti-CVB4 mouse mAb	Millipore (MAB941)	FFPE (AKF)	Pepsin		DAKO Envision HRP	Does not work in FFPE
SR/NGM	L Miao anti-CVB3 vp1 mouse mAb	L Miao	FFPE (AKF)	1mM EDTA pH8	1ug/ml	DAKO Envision HRP	Works well in infected cells, see table. Can seesignal in infected heart in the same region as Dako vp1, but immune cells are also positive in this and other tissue controls. In pancreas of T1D see occasional positive endocrine cell and positive immune cells.
SR/NGM	L Miao anti-Polio vp1 mouse mAb	L Miao	FFPE (AKF)	1mM EDTA pH8	2ug/ml	DAKO Envision HRP	Work well in infected cells, see table. Positive cells in infected heart, but in control pancreas stains endocrine cells and both IDIs and ICIs in T1D.



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Task 2: EV antibodies

- We now have information on 17 different EV antibodies (2 more from Rick in Houston)
- The most commonly used EV antibody is Dako vp1 (5D8/1)
- Most antibodies work in acutely-infected cell lines and recognise a broad spectrum of EV serotypes
- However, the majority that we have tested do not work in confirmed EV-infected neonatal FFPE heart tissue




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Task 2: Acute Models of EV infection

LABORATORY	Details	Source	FFPE/ Frozen	Antibodies trialled	Conditions	Results	Comments
SR/NGM/AKF	CVB1-5 acutely infected Vero cells and uninfected controls	Glasgow	FFPE	anti-enterovirus (5D8/1)	1mM EDTA pH8; 1/750-1/2000. Envision HRP Detection	Clear positive staining in CVB1-5, uninfected negative	Good for initial screening and optimisation of antibodies
				kdf anti-vp1	1mM EDTA pH8; 1/800. Envision HRP Detection	Clear positive staining in CVB1-5, uninfected negative but some background	
SR/NGM/DH	CVB-infected neonatal mice and uninfected controls	Plymouth	FFPE	anti-enterovirus (5D8/1)	1mM EDTA pH8; 1/5000. Envision HRP Detection	Clear positive staining in cells previously identified by enterviral specific ISH as being positive	Various CVB serotypes.These samples are around 20 years old
				kdf anti-vp1	1mM EDTA pH8; 1/800. Envision HRP Detection		
SR/NGM/AKF	Confirmed CVB-infected neonatal heart and pancreas, uninfected controls	Glasgow	FFPE	anti-enterovirus (5D8/1)	1mM EDTA pH8; 1/500. Envision HRP Detection	Clear positive staining in hearts and pancreas. Uninfected occasional wash of background in some hearts. Positive staining similar to EV specific ISH	Various CVB serotypes, not all known.These samples are around 20 years old and there is limited avaliability
				kdf anti-vp1	1mM EDTA pH8; 1/400. Envision HRP Detection	Clear positive staining in hearts and pancreas. Uninfected frequent wash of background in hearts	
SR/NGM	VIROL TMAs	HH/MO	FFPE	anti-enterovirus (5D8/1)	1mM EDTA pH8; 1/1000. Envision HRP Detection	See TMA table	Good for initial screening and optimisation of antibodies
				kdf anti-vp1	1mM EDTA pH8; 1/800. Envision HRP Detection	See TMA table	
				L Miao anti-CVB3 vp1	1mM EDTA pH8; 1ug/ml. Envision HRP Detection	See TMA table	
				L Miao anti-Polio vp1	1mM EDTA pH8; 1ug/ml. Envision HRP Detection	See TMA table	
				Pan-enterovirus Blend	10mM Tris 1mM EDTA pH9; 1/3. Envision HRP Detection	See TMA table	
Tampere	CBV1-6, CAV9 and 16, echo3, 4, 6, 9, 11 and 30, ent 71, and polio3-infected cells and uninfected controls		FFPE	All antibodies listed in table 2	Listed in table 2		Also frozen sections available
Tampere	CBV3-infected mice and uninfected controls		FFPE				



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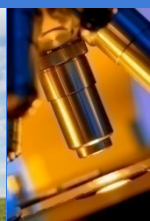

Task 2: Acute Models of EV infection

- EV-infected cell lines
- EV-infected human islets
- Coxsackie-infected mice
- Coxsackie-infected FFPE human heart

Useful for optimising EV antibody staining conditions and defining serotype recognition



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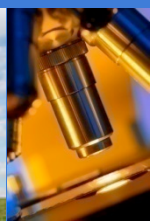

Task 2: Persistent Models of EV infection

- Human islets infected with persistent EV's – Gun Frisk
- Tissue from mice with a persistent EV infection – Nora Chapman
- Persistently-infected HeLa – Antonio Toniolo
- Others???

Important to determine which EV antibodies can detect persistent infections, assist in the development of techniques to optimise detection of viral RNA and to examine which 'viral footprint' markers are expressed under these conditions




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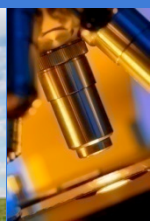

Task 3: 'Viral Footprint'

VIRUS

↓

PATHOGEN RECOGNITION RECEPTORS; PKR, MDA5, OAS, RIGI

↓

TYPE 1 INTERFERONS; IFN alpha and IFN beta

CLASS I MHC

CHEMOKINES; CXCL10 (Sarkar, Diabetes)

ADHESION MOLECULES; ICAM1

OTHERS; Survivin, Ki67



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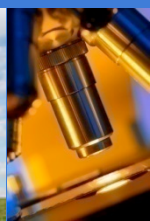
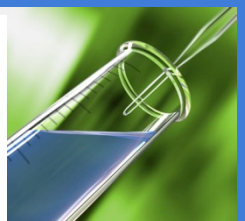


Thank you to all who contributed to these databases!

I look forward to a productive and stimulating 2012

JDRF IMPROVING LIVES. CURING TYPE 1 DIABETES.



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