Great Expectations
[PANDEMIC INFLUENZA]

September 2010

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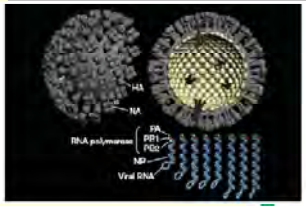
Themes

- Influenza
- Biology of pH1N1
- "The Lab"
- Surveillance
- The Future
- Questions

Structure & Function

Segment	Size	Peptide(s)	Function
1	2341	PB2	RNA polymerase subunit
2	2341	PB1	RNA polymerase subunit
3	2233	PA	RNA polymerase
4	1778	HA	Haemagglutinin
5	1565	NP	Nucleoprotein
6	1413	NA	Neuraminidase: release of virus
7	1027	M1	Matrix protein: major component of virion
		M2	Integral membrane protein - ion channel
8	890	NS1	Anti-interferon protein/virulence factor.
		NS2	"Virulence factor"



Subtypes of Influenza A

H1 - swine, human	N1 - human
H2 - human	N2 - human
H3 - human	N3 - avian
H4 - avian	N4 - avian
H5* - avian, (human)	N5 - avian
H6 - avian	N6 - avian
H7* - equine,avian, (human)	N7 - equine
H8 - avian	N8 - equine
H9 - avian, (human)	N9 - avian
H10 – H16 - avian	

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What's in a Name !!

- 15th April – "Swine-Origin Influenza Virus S-OIV"
- 22nd April – "Swine influenza A (H1N1)"
- 26th April – "Human swine influenza (H1N1)"
- 27th April – "A/H1N1 influenza"
- 22nd May – "Novel Influenza A (H1N1)"
- 1st July – Pandemic (H1N1) 2009
- ? Seasonal/Pandemic Influenza

Pandemic (H1N1) A/California/07/2009

A/Brisbane/59/07-like [H1N1]

Type | Geographical Origin | Isolate Number | Year of Isolation | HA component | NA component

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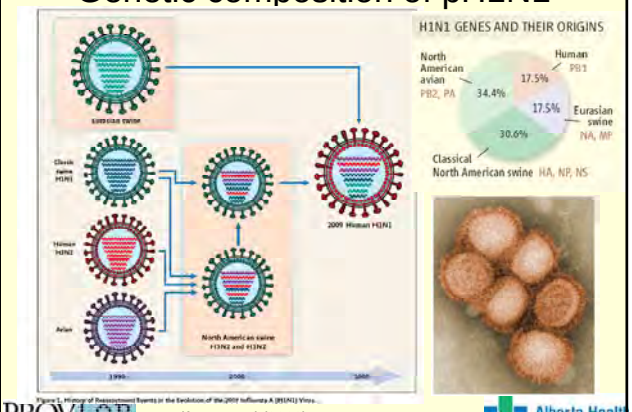
Genetic composition of pH1N1



Trifonov et al (2009) NEJM

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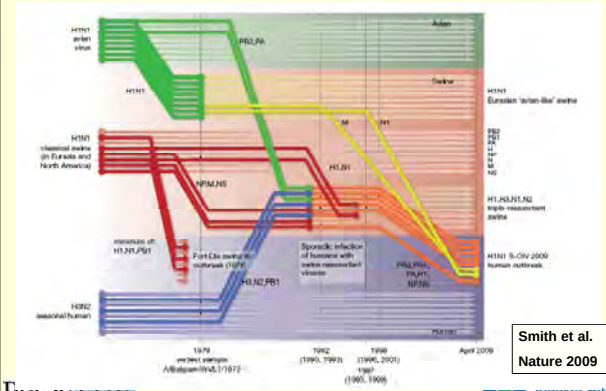
Origin of Pandemic Influenza



Smith et al. Nature 2009

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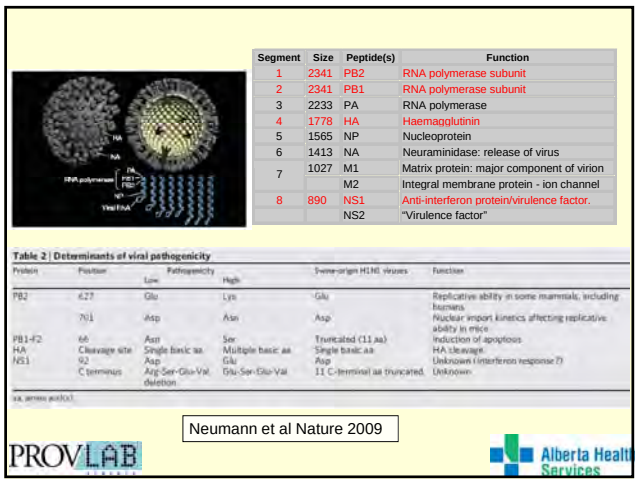

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		NS2	"Virulence factor"

Table 2 | Determinants of viral pathogenicity

Protein	Position	Pathogenicity: Low	Pathogenicity: High	Swine-origin H1N1 viruses	Function
PB2	627	Glu	Lys	Glu	Replicative ability in some mammals, including humans
	701	Asp	Asn	Asp	Nuclear import kinetics affecting replicative ability in mice
PB1-F2	66	Asn	Ser	Truncated (11 aa)	Induction of apoptosis
HA	Cleavage site	Single basic aa	Multiple basic aa	Single basic aa	HA cleavage
NS1	92	Asp	Glu	Asp	Unknown (interferon response?)
	C terminus	Arg-Ser-Glu-Val	Glu-Ser-Glu-Val	11 C-terminal aa truncated	Unknown

Neumann et al Nature 2009

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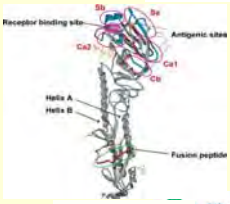
How different is pH1N1?

- Low mortality 0.02% vs 2.4% for Spanish influenza and 0.2% for seasonal
- Mainly a "mild" upper respiratory tract infection – no sequelae
- Affects young vs old
- Seniors appear to be resistant to illness.
- Animal models seem to confirm mild illness
- Risk factors
  - morbid obesity (>40 kg) OR-7.6
  - Pregnancy – OR 7.4 overall and 13.2 in 3rd trimester



Bouvier (2008) Vaccine

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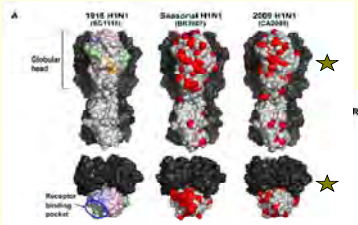
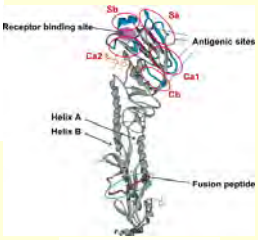


Igarashi et al PLos ONE 2010



Bouvier (2008) Vaccine

Table 1. Amino acid similarity in the HA antigenic sites among recent seasonal H1N1 (BR2007), 2009 H1N1 (CA2009), and 1918 H1N1 (SC1918).

Antigenic sites	No. amino acids involved	No. of amino acids identical to SC1918	
		BR2007	CA2009
Sa	13	8	12
Sb	12	4	10
Ca	19	13	13
Cb	6	2	5

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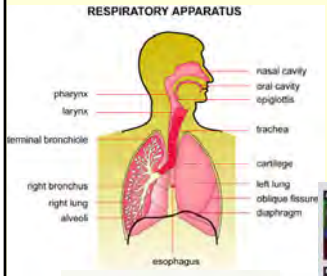
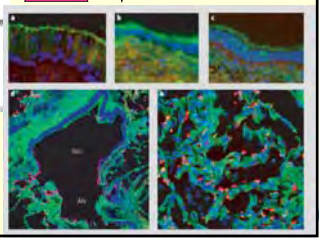
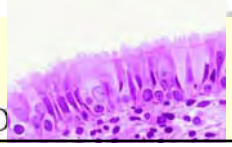
Distribution of Receptors in the Respiratory Tree



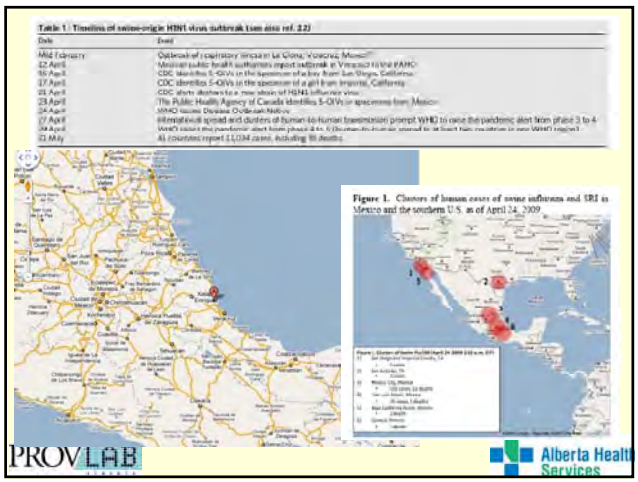
α2,6

α2,3

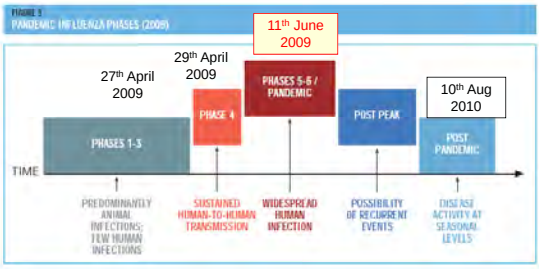




PRO



Timeline of Phases



Pandemics of Influenza A

Year	Colloquialism	Subtype	Deaths x10^6	Origin
1889		H2N2	6	Europe
1898		H3N2	0.5	Europe
1918	Spanish flu	H1N1	20-40	Europe
1957	Asian flu	H2N2	4	Asia
1968	Hong Kong flu	H3N2	2	Asia
1977	Russian flu	H1N1	?	Asia /lab
2009	**Swine-origin**	**H1N1**	**18 398 or so (Lab confirmed)**	**Mexico**

JS Oxford: Rev Med Virol:2000:10;119-33

Laboratory Role

Laboratory Role

- Detection & characterization of pH1N1
- Surveillance
  - Antiviral resistance
  - Antigenic Drift
  - Other respiratory viruses
- Lab-Confirmed Case Line List
- Studies !!

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Detection of pH1N1

Culture

- Takes 3-10 days
- Poorly sensitive (approx <30% relative to PCR)
- Sample has to be fresh for virus to be viable

Serological

Hemagglutination inhibition assay (HAI)
- Cross reactive between similar subtypes
- Highly labour intensive
- For optimal interpretation requires acute & convalescent bloods

Microneutralization Assay
- Require paired bloods (min 3 weeks apart)
- Current data shows that cross-neutralization to related subtypes can occur
- Different strains may give different results
- No data to definitively link this finding to immunity

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Detection by Point-of-Care

•Antigen Assays
- Point of Care
- Direct Fluorescent Antigen

➢Advantages
- •Quick
- •Can be done by "anyone"
- •Various samples

➢Disadvantages
- •Sens <50%
- •Cannot tell if pandemic or seasonal subtype
- •Confirmation is a must

Table 1. Analytic Performance of Rapid Influenza Antigen Tests, Compared with (Luminex) Reverse-Transcriptase Polymerase Chain Reaction

Test	Sensitivity, % (95% CI)	Specificity, % (95% CI)	PPV, %	NPV, %
BD Directigen EZ Flu A+B	46.7 (34.6–59.1)	100 (86.2–100)	100	89.6
BinaxNOW Influenza A&B	39.3 (27.1–51.0)	100 (86.2–100)	100	88.2
QuickVue Influenza A+B Test	53.3 (40.9–65.4)	100 (86.2–100)	100	90.8

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Molecular Testing

Advantages:
- Highly sensitive (10-100 cps)
- Rapid Turnaround Time
- Virus does not have to be viable
- Variety of sample types can be used
- Can subtype directly from sample
- High through put
- Quantification a possibility
- Variety of various viruses can be detected from the same sample
- Currently recommended technique to detect influenza

Disadvantages
- Only works if you know what to look for
- Mutations in crucial regions can give false negative results
- High staff expertise required
- **Are low levels infectious !!**
- Awareness of pitfalls
- Specialized facilities required (clean air, no contamination, etc)
- Specialized supplies required
- Cost
- Testing only available in specialized centres
- Can result in restricted testing

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Molecular Testing

- Respiratory Viral Panel (Luminex)
  - Detects influenza A&B, RSV, parainfluenza gp, coronaviruses, adenovirus, human metapneumovirus, rhino/enterovirus
  - Subtypes seasonal influenza A
  - 48 hrs to result
- Detects multiple viral agents (co-infections)
- Surveillance for other agents
- Able to subtype seasonal influenza but not pandemic

- RT-PCR for influenza A
  - Detects all subtypes of influenza A
  - 12 hrs to result
  - Highly sensitive
- Better sensitivity for influenza A
- Excellent screening assay for community patients

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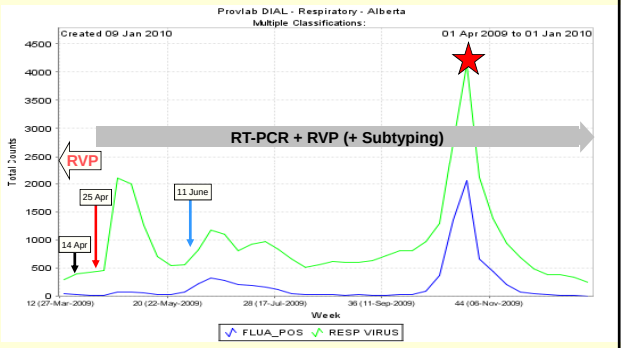
Distribution of Specimens & Viruses by Month (data from DIAL)



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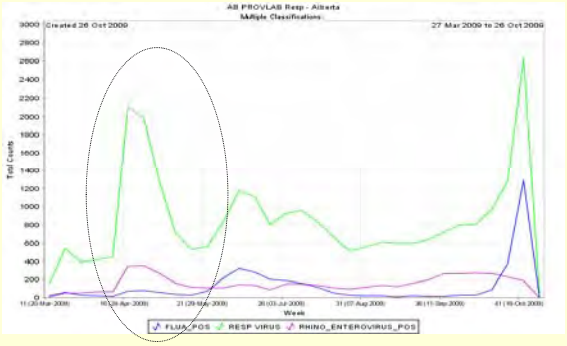

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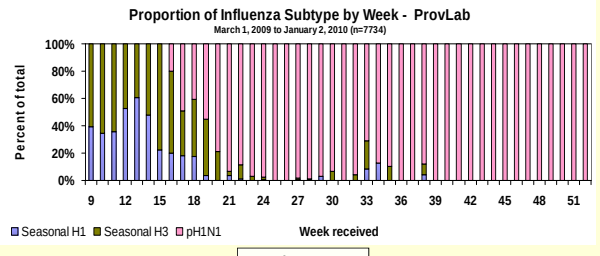
Proportion of Influenza Subtype by Week - ProvLab

March 1, 2009 to January 2, 2010 (n=7734)



Data from DIAL

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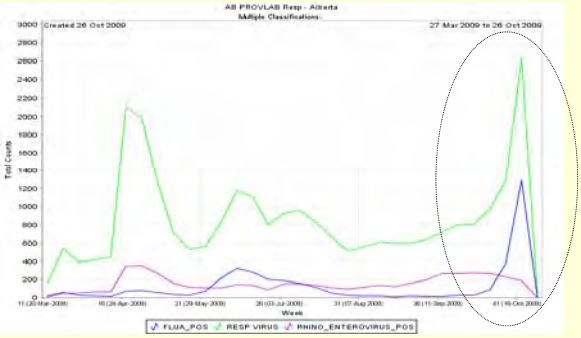
Lessons Learned from “1st Wave”

- Lots of samples received for testing
  - Lots of overtime
  - **Problems with prioritization**
  - Too much of the wrong order of testing
  - Frequent change in lab testing protocols was confusing to stakeholders
- Lab rapidly overwhelmed
- **Insufficient equipment (or arrived too late !!)**
- Too many meetings of questionable value
- Mixture of seasonal & pandemic required subtyping resources

Distribution of Specimens & Viruses By Month

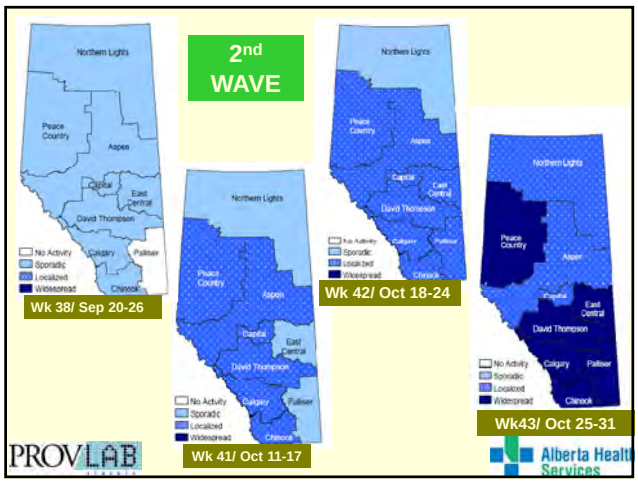
(data from DIAL)

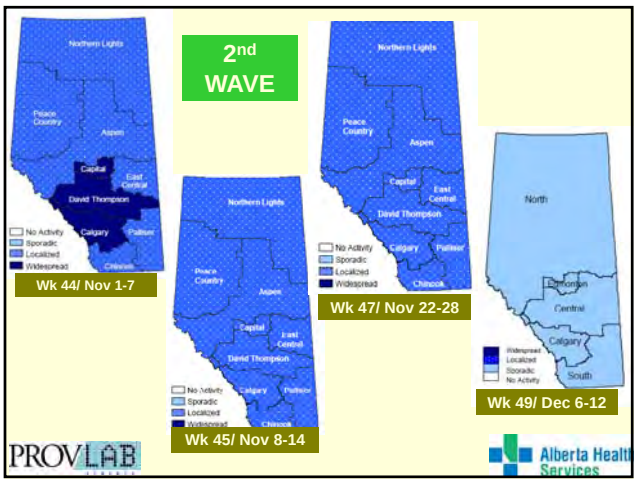


Surveillance Picture

Data from GoA Website

http://www.health.alberta.ca/health-info/influenza-evidence.html#Archive





Seasonal Influenza vs pH1N

Government of Alberta

(data provided by Dr. Pamela Steppan)

- The chart on the right shows the number of hospitalizations and deaths per year primarily attributed to influenza.
- ***It is important to note that the deaths and hospitalizations due to pH1N1 still need to be further classified by primary cause, as current numbers reflect cases both caused by H1N1 and those who are hospitalized or die due to other reasons.**

YEAR	Deaths	Hospitalized
2002	28	535
2003	11	784
2004	7	357
2005	29	451
2006	15	440
2007	13	260
2008	18	321
2009 pH1N1 only	71	1,278

Lab Investigated Outbreaks

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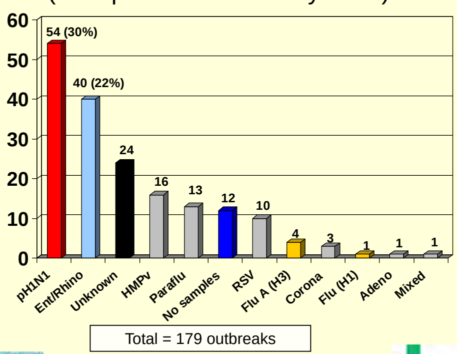
Nos of outbreaks by Agent

(1st April 2009 to 30 May 2010)



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By Facility

	Facility Type				
Agent	**School**	**LTC/Lodge**	**Acute**	**Other**	**Total**
pH1N1	35	8*	5*	6	54
Ent/Rhino	4	32	3	1	40
Unknown	1	21	2		24
HMPv		15		1	16
ParaFlu		13			13
RSV		10			10
Flu H3		4			4
Corona		2	1		3
Flu H1	1				1
Adeno	1				1
Mixed	1				1
Total	43	105	11	8	168

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Outbreaks - Observations

- Can serve as sentinel events for the emergence of a new variant
- Lab confirmation helps with characterization of new variants or viruses
- No agent in 24/168 (15%) outbreaks
  - Missed diagnosis
  - New virus (es) ?
  - Inadequate nos of samples or sampling technique?
  - Samples collected late in outbreak ?
- Are bacterial pathogens being missed ?

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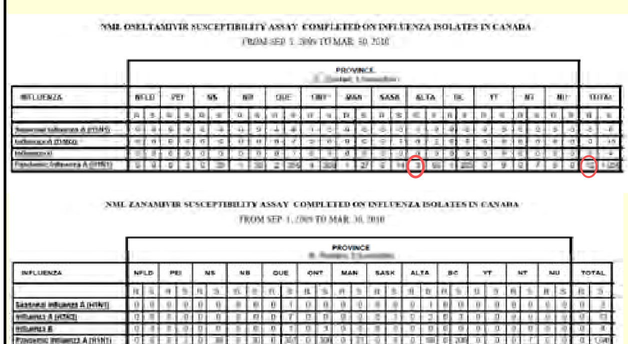
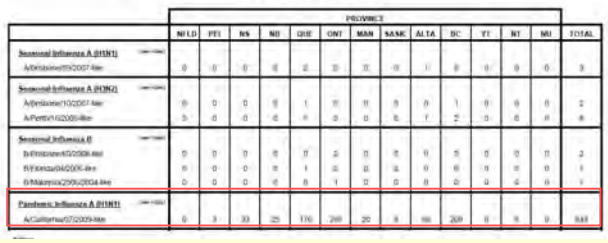


PROVLAB **Data from Dr. Yan Li @ NML** Alberta Health Services

NML STRAIN CHARACTERIZATION COMPLETED ON INFLUENZA ISOLATES IN CANADA

FROM SEP 1, 2009 TO MAR. 31, 2010



Pandemic Influenza A (H1N1):
Of the 843 pandemic influenza A (H1N1) viruses characterized, 839 (99.5%) were antigenically related to A/California/7/2009, which is the pandemic reference virus selected by WHO as the 2009 H1N1 vaccine. Four viruses
(0.5%) tested showed reduced titer with antisera produced against A/California/7/09.

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Data from Dr. Yan Li @ NML

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The Future

- We should plan for the next Pandemic !!
- Confusion between seasonal pandemic and pandemic pandemic
- Monitoring for antigenic drift
- Monitoring for antiviral resistance
- Vigilance in changing spectrum of clinical illness
- We need more resources !!
- We were fortunate this time – may be less so next time
- The next one could still occur in our backyard

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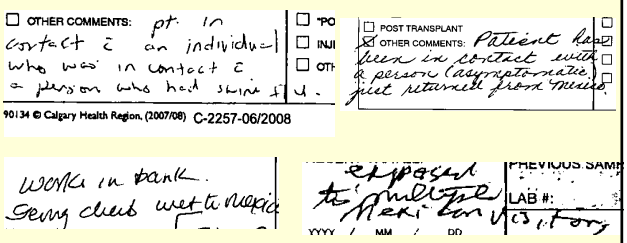
Reasons for Testing - 1st Wave



Other - PATIENT OF MEXICAN DESCENT, NO RECENT TRAV
No
7:46 PM

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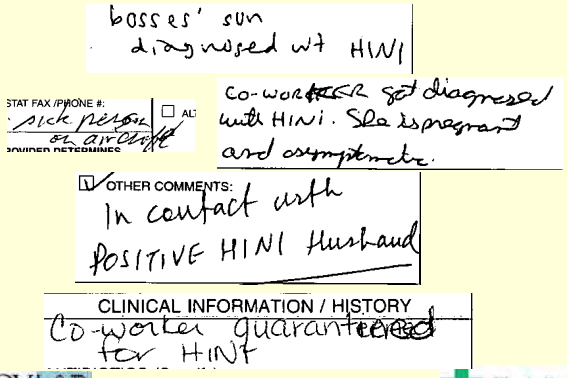
Excuses 2nd Wave



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Questions

- Acknowledgements
  - Dr. Marie Louie
  - Dr. Bonita Lee
  - Jennifer May-Hadford
  - DIAL Team
  - ProvLab Staff
  - IPC Team
  - CD Teams
  - CLS, DynaLife and Rural Labs

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