LABORATORY STANDARDS AND PROCEDURES WORKGROUP

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Laboratory Standards & Procedures Workgroup Charge

Define and implement a mechanism for the periodic review and assessment of

1. The conditions included in the uniform panel
2. Laboratory procedures utilized for effective and efficient testing of the conditions included in the uniform panel.
3. Infrastructure and services needed for effective and efficient screening of the conditions included in the uniform panel

New Project 1

1. Laboratory procedures: Explore the role of next generation sequencing in newborn screening

- Screening is currently based on phenotypic data. How do we accumulate the data to identify correlation between phenotypic & genotypic data?
- Are there conditions for which sequencing is the only screening method?
- What do you gain/lose from NGS?
- Which data do you report? VUS? Carrier status?
- What new infrastructure needs to be built for NGS?

New Project 2

2. Infrastructure and services:
    - Review data related to testing (Timeliness 1.0)
    - What are the implications of earlier specimen collection (<24 hrs)?
    - What are the unforeseen consequences and costs of timeliness?

Agenda

1. Welcome & roll call
2. Review charge & new projects
3. Role of whole genome sequencing in newborn screening
   a. Overview of APHL Molecular Subcommittee – Michele Caggana –15 min
   b. Next Gen Sequencing in a state NBS Program – Mei Baker – 15 min
4. Timeliness –
   a. Early specimen collection – Lisa Feutchbaum - 20 min
   b. Unintended consequences and costs of timeliness – Marci Sontag - 20 min

Overview of APHL Molecular Subcommittee – Michele Caggana

1. History
2. Molecular Quality Improvement Program
3. NBS Molecular Workshops
4. Molecular Assessment Program
5. NBS Molecular Resources Website
6. Paradigm for NBS Molecular Pilots
7. NextGen sequencing meeting for the NBS community - APHL/CDC, Q1 2017

Next Generation Sequencing in NBS: Are we there yet?

Mei Baker, MD, FACMG

Co-Director, Newborn Screening Laboratory at WSLH
Professor, Department of Pediatrics
University of Wisconsin School of Medicine and Public health

SACHDNC Laboratory Procedures and Standards Subcommittee
May 9, 2016

Progression of CF NBS Tests

IRT →	IRT/DNA →	IRT/DNA* →	IRT/DNA/DNA**
	(F508del)	(CFTR-23)	(CFTR > 200)
1979 →	1991	→ 2003	→ 2012-16

*With IRT/DNA, 10 heterozygote carriers are detected for every CF infant diagnosed.

**IRT/NGS algorithm applying CFTR2 knowledge and next generation sequencing capability, which may be a “game-changer”.

CFTR2 Mutation List History

	V1 4/10/2012	V2 7/22/2013	V3 2/27/2015	V4 8/13/2015
Number of Patients	35,312	39,696	39,696	88,664
CF-causing	123	175	179	242
Varying Clinical Consequence	15	12	12	19
Non CF-causing	5	10	10	12
Unknown Significance	15	6	6	3
Total	158	203	207	276

Prospective Study

- **<u>SPECIFIC AIM 1</u>.** We will further modify the established Illumina NGS method to expand *CFTR* mutation panel up to 250 CF-causing mutations.

- **<u>SPECIFIC AIM 2</u>.** We will demonstrate that the IRT/NGS CF screening protocol can significantly reduce false positive results caused by identification of CF heterozygote carrier infants in a real-world NBS environment.

Early specimen collection in California – Lisa Feutchbaum

1. We used California population-level data to determine whether early specimens (collected from 12 to 23 hours) would also be considered satisfactory based on screening performance.
2. CA analyzed for false-negative and false-positive rates in four disease categories:
   - metabolic disorders detectable by tandem mass spectrometry (MS/MS);
   - congenital adrenal hyperplasia (CAH);
   - congenital hypothyroidism (CH);
   - initial immune reactive trypsinogen (IRT) for cystic fibrosis (CF).
3. We compared the rates between the early-collection group (12 to 23 hours) and the standard-collection group (24 to 48 hours).

Early specimen collection in California – Lisa Feutchbaum

4. Conclusion:
   - No significant difference of false-negative rate was detected between the two collection-timing groups.
   - Early specimens had a significantly higher false-positive rate for CH (0.10 vs. 0.01%) and IRT (1.85 vs. 1.54%) but a lower false-positive rate for MSMS metabolic disorders (0.11 vs. 0.18%) and CAH (0.10 vs. 0.14%).

Tang, H. et al. Damaged goods?: an empirical cohort study of blood specimens collected 12 to 23 hours after birth in newborn screening in California. Genetics in Medicine (2016) 18, 259–264.

UNINTENDED CONSEQUENCES OF TIMELINESS

OBJECTIVES

Provide overview of NBS timeliness concerns

Discuss methods for collecting/analyzing data

Review state data provided (NY, MN, WI, IA) and discuss implications

Discuss challenges and next steps

Concerns

- Pre-Analytic
  - Less time to consult parents in hospital prior to screen
  - Collecting specimens from NICU/VLBW newborns
- Analytic
  - Repeat testing due to more out-of-range/borderline results
  - Asking for additional specimens due to more out-of-range/borderline results
- Post-Analytic
  - Increase in missed cases (false negatives)
  - Increase in presumptive positives (false positives)

Methods

- Report proportion of presumptive positives for IRT and TSH by age/time of collection
  - Correlation of analyte vs. age at time of collection
  - Proportion of presumptive positives by age of collection
- Look at borderline cases separately
- Remove VLBW babies (<1000g) from analysis as they tend to have atypical values

Minnesota—IRT

	Age at Time of Collection (hrs.)	Total IRT results reported	Total Out-of-Range (PP+ Borderline)	PP n (%)	Borderline n (%)	True +	PPV
2015	0:00 – 11:59	381	15	4 (1.05%)	11 (2.89%)	1	6.7%
	12:00 – 23:59	175	1	1 (0.57%)	0 (0%)	0	0.0%
	24:00 – 29:59	47105	174	174 (0.37%)	0 (0%)	17	9.8%
	30:00 – 47:59	19439	76	75 (0.39%)	1 (0.01%)	8	10.5%
	≥ 48:00	5173	15	14 (0.27%)	1 (0.02%)	3	20.0%
2014	0:00 – 11:59	330	9	3 (0.91%)	6 (1.82%)	0	0.0%
	12:00 – 23:59	175	3	2 (1.14%)	1 (0.57%)	0	0.0%
	24:00 – 29:59	40803	137	137 (0.34%)	0 (0%)	17	12.4%
	30:00 – 47:59	18460	36	36 (0.2%)	0 (0%)	2	5.6%
	≥ 48:00	4700	14	13 (0.28%)	1 (0.02%)	1	7.1%

Next Steps

- Need more data (i.e. how many babies are being called out as abnormal by age of collection)
- Identify some analytes that may be called out as *normal* when drawn early
- Need to monitor for missed cases
- Pre-analytic concerns and measures