Hemoglobin Variant Reporting in Newborn Screening

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NEWBORN SCREENING

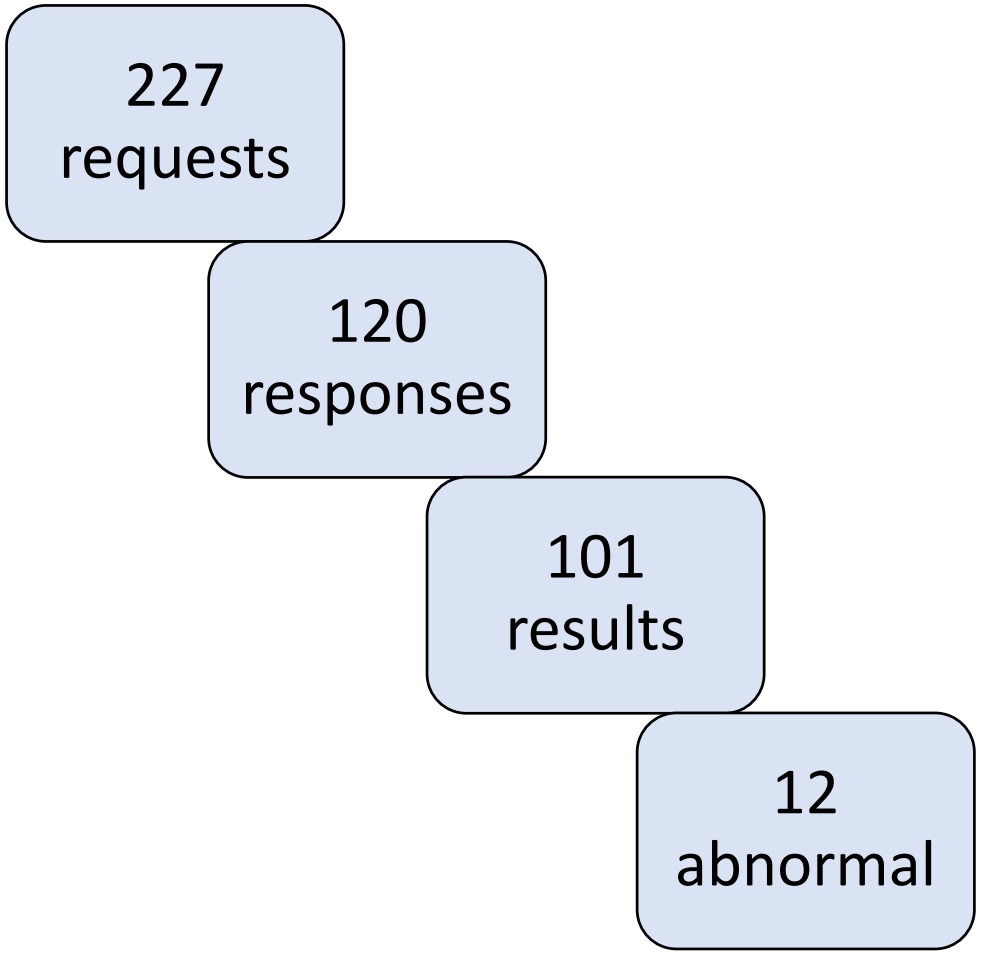
Hemoglobinopathies Overview

- Hemoglobin disorders include sickle cell disease, alpha thalassemia, and beta thalassemia
- Prevalence of Hgb carriers between 5% and 20%
  - African American – 10% with sickle cell trait
- Newborn Screening began testing in the 1970s
- Utah began screening in 2001

Utah Hemoglobin Newborn Screening

- First screen – isoelectric focusing
- Second screen – isoelectric focusing and HPLC if IEF abnormal
- Utah reports FAU – Carriers of unidentified variants
  - Recommend complete blood count (CBC) and hemoglobin evaluation using HPLC between 6-9 months of age

Quality Improvement Study: Cases in 2017 identified with FAU



Results

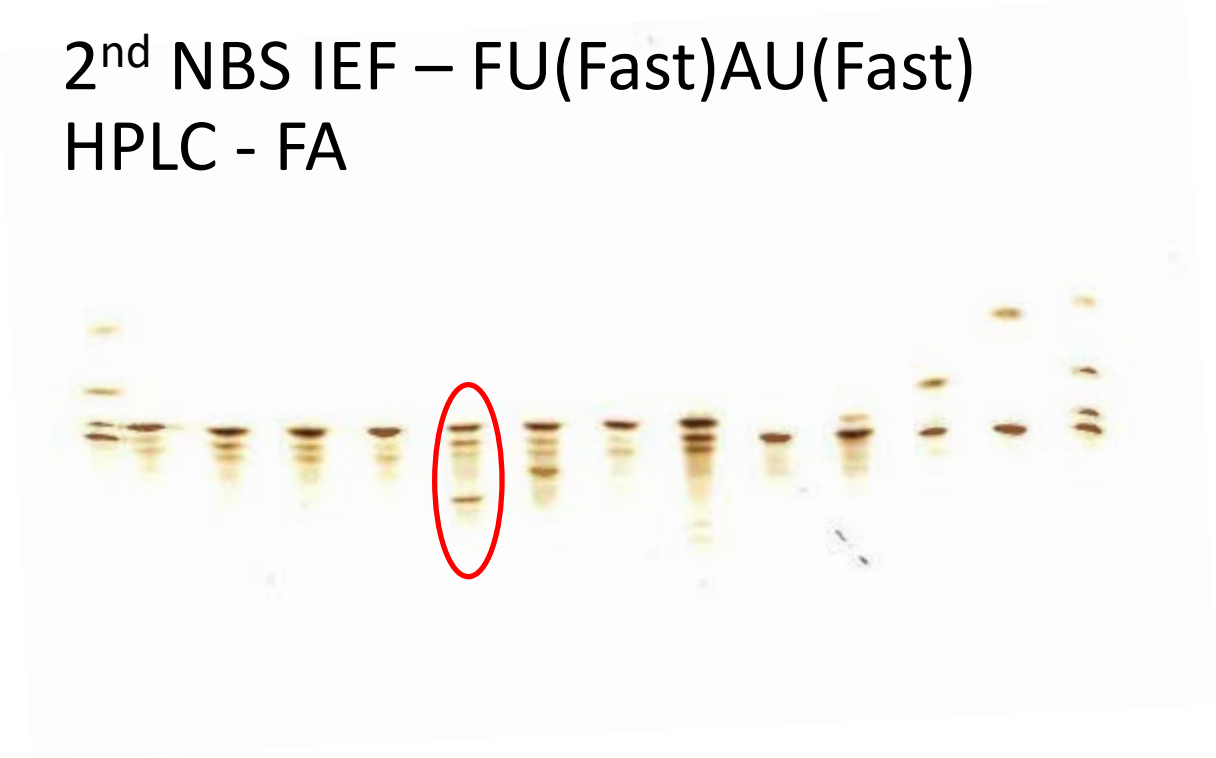
- 12 abnormal
  - Hb N-Baltimore
  - Hb I
  - Hb J-Baltimore
  - Hb J-Toronto
  - Hb J-Broussaid
  - Hb Manitoba
  - Hb Other (44%) – no further testing completed to identify
  - Hb Other (45.4%) – no further testing completed to identify
  - Hb Other (23.1%) – no further testing completed to identify
  - CBC – microcytosis referral to Hematology
  - 2 cases: persistent of fetal hemoglobin

Hemoglobin I

1st NBS IEF – FU(Fast)AU(Fast)

2nd NBS IEF – FU(Fast)AU(Fast)

HPLC - FA



Confirmatory/Diagnostic Testing Results

CBC	Results	Ref range
Hematocrit	34.8	33-39
MCV	79.8	70-86
MCH	26.6	23-31

Hemoglobin	Results (%)	Ref Range
Hemoglobin A	75.8% (L)	86.1-97.2
Hemoglobin A2	2.3%	1.9-3.5
Hemoglobin F	1.4%	0.6-11.6
Hemoglobin Other	20.5% (H)	0-0

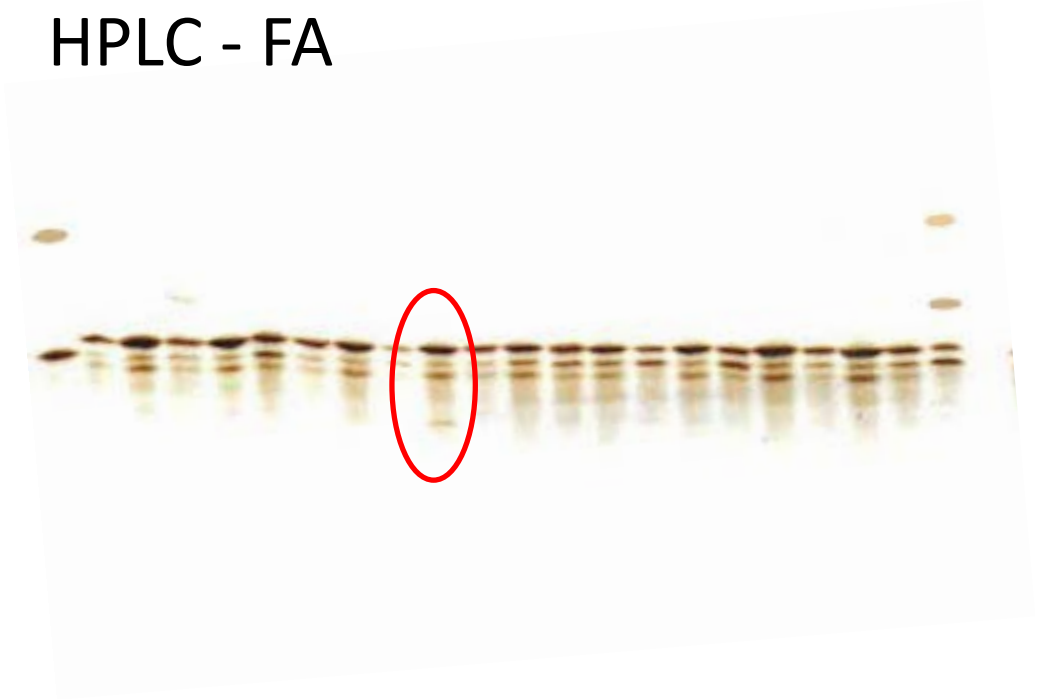
DNA results – *HBA2*: c.49A>G; Lys16Glu – likely benign

Hemoglobin N-Baltimore

1st NBS IEF – FU(Fast)A

2nd NBS IEF – FU(Fast)A

HPLC - FA



Confirmatory/Diagnostic Testing Results

CBC	Results	Ref range
Hematocrit	34.1	33-39
MCV	83.4	70-86
MCH	27.9	23-31

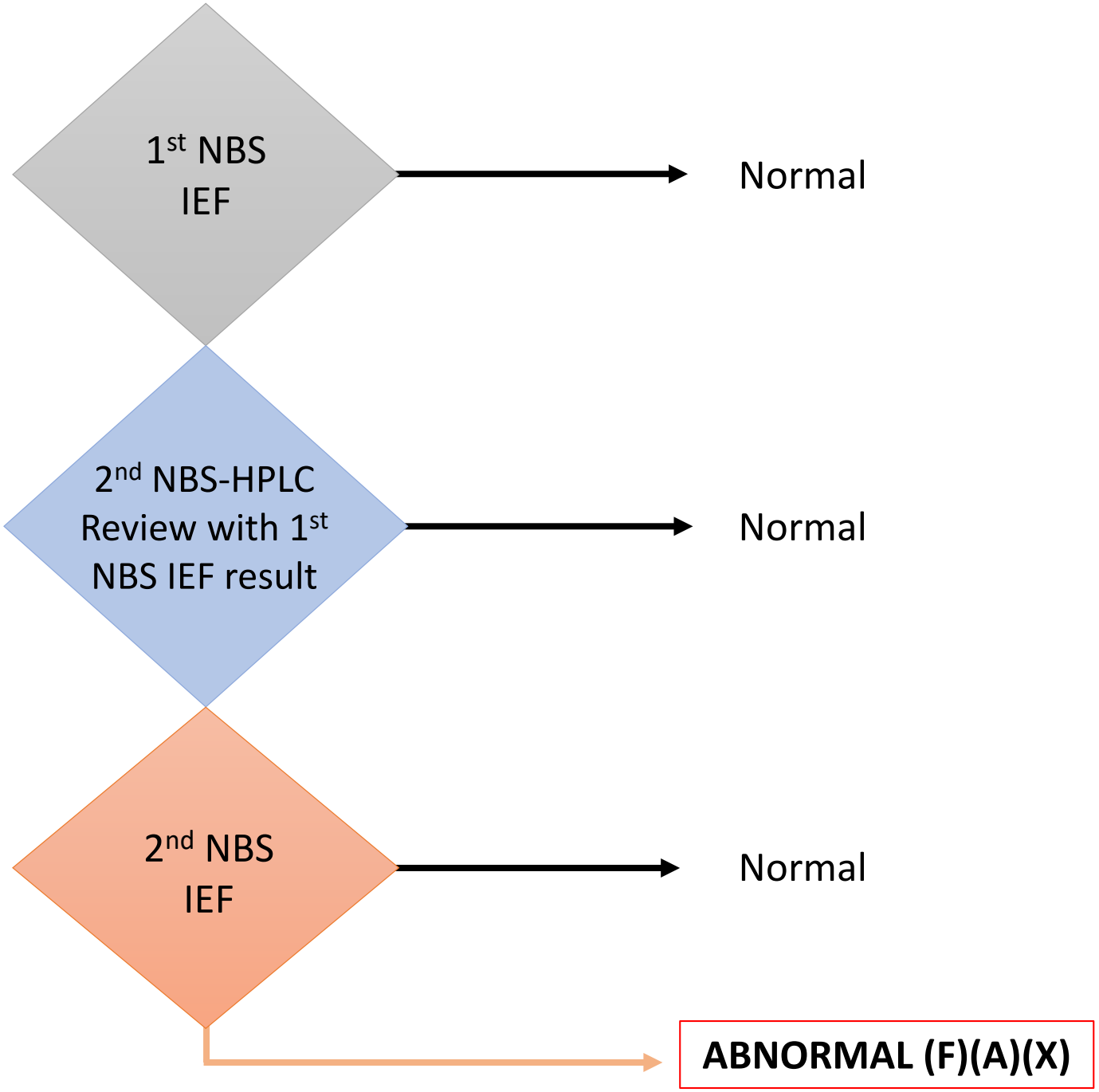
Hemoglobin	Results (%)	Ref Range
Hemoglobin A	49.7% (L)	85-97
Hemoglobin A2	2.6%	1.9-3.5
Hemoglobin F	3.7%	0-8.5
Hemoglobin Other	44% (H)	0-0

DNA results – *HBB*: c.286A>G; p.Lys96Glu – likely benign

Conclusion

- Most Unidentified Hemoglobin Variants are benign, **however not all**
  - Associated with aberrant HbA1c values affecting diagnosis/treatment of diabetes
  - Hemolytic anemia/splenomegaly – Ex. Hb Brevedent
  - Hemolytic mild chronic anemia – Ex. Hb I-Toulouse
- Hb “Other” persistent at the 9 month period

Is HPLC a suitable primary screening method?


1st NBS
IEF
Normal
2nd NBS-HPLC
Review with 1st
NBS IEF result
Normal
2nd NBS
IEF
Normal
ABNORMAL (F)(A)(X)

Considerations

- DNA analysis to confirm variant to determine clinic symptoms
- New updates to Utah NBS process
  - IEF as first screen
  - HPLC as second screen for abnormal results
- Alternatives
  - Repeat IEF on second screen if results are divergent
  - Sequencing
- Report obvious FAU bands with same recommendations