

NAT Algorithm Design

Review of Presentations and Discussion

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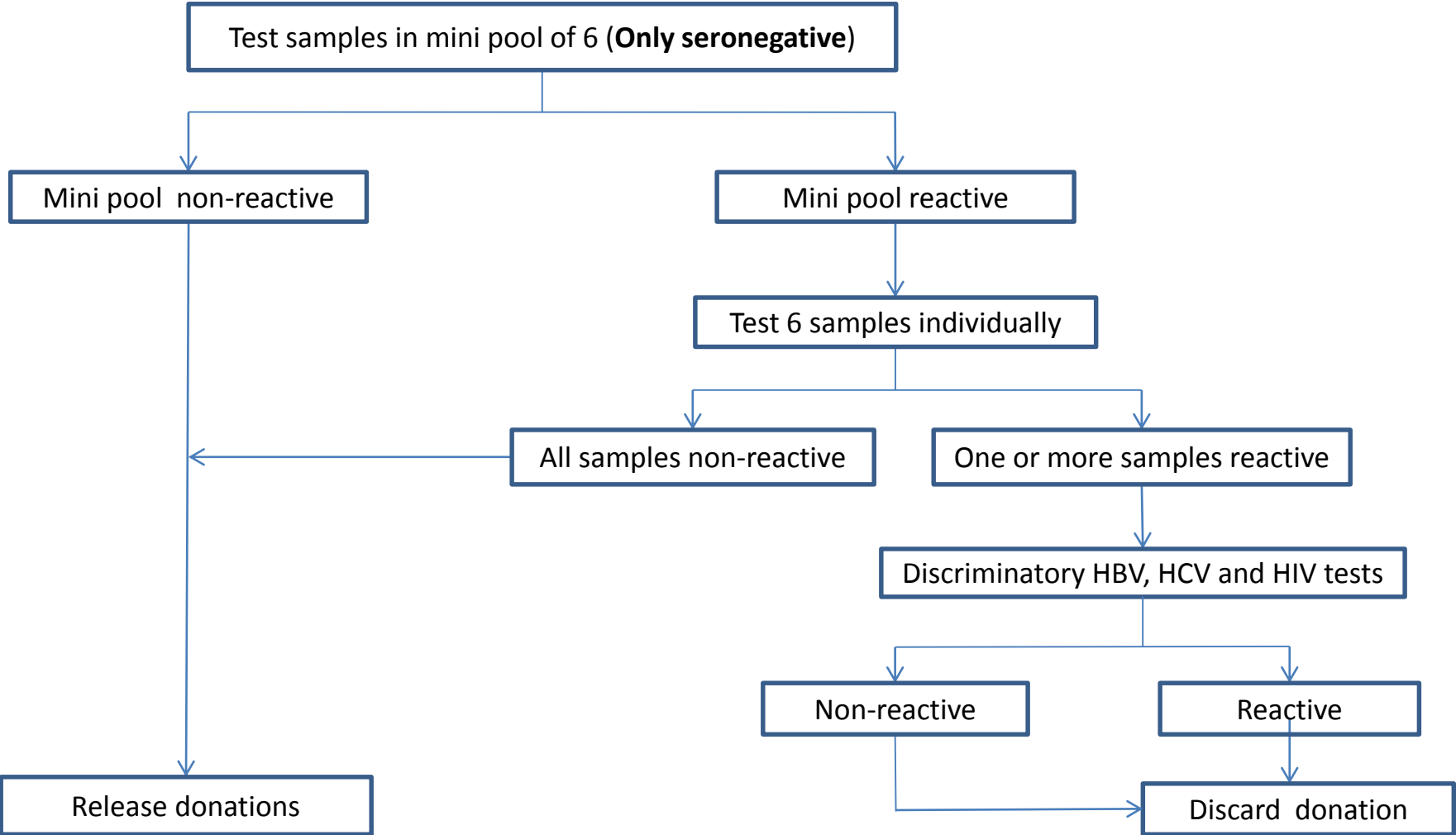
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2. Irish Blood Transfusion Service, Dublin, Ireland
3. South African National Blood Service, Johannesburg, South-Africa
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6. Thai Red Cross, Bangkok, Thailand
7. Lelie Research, Paris, France

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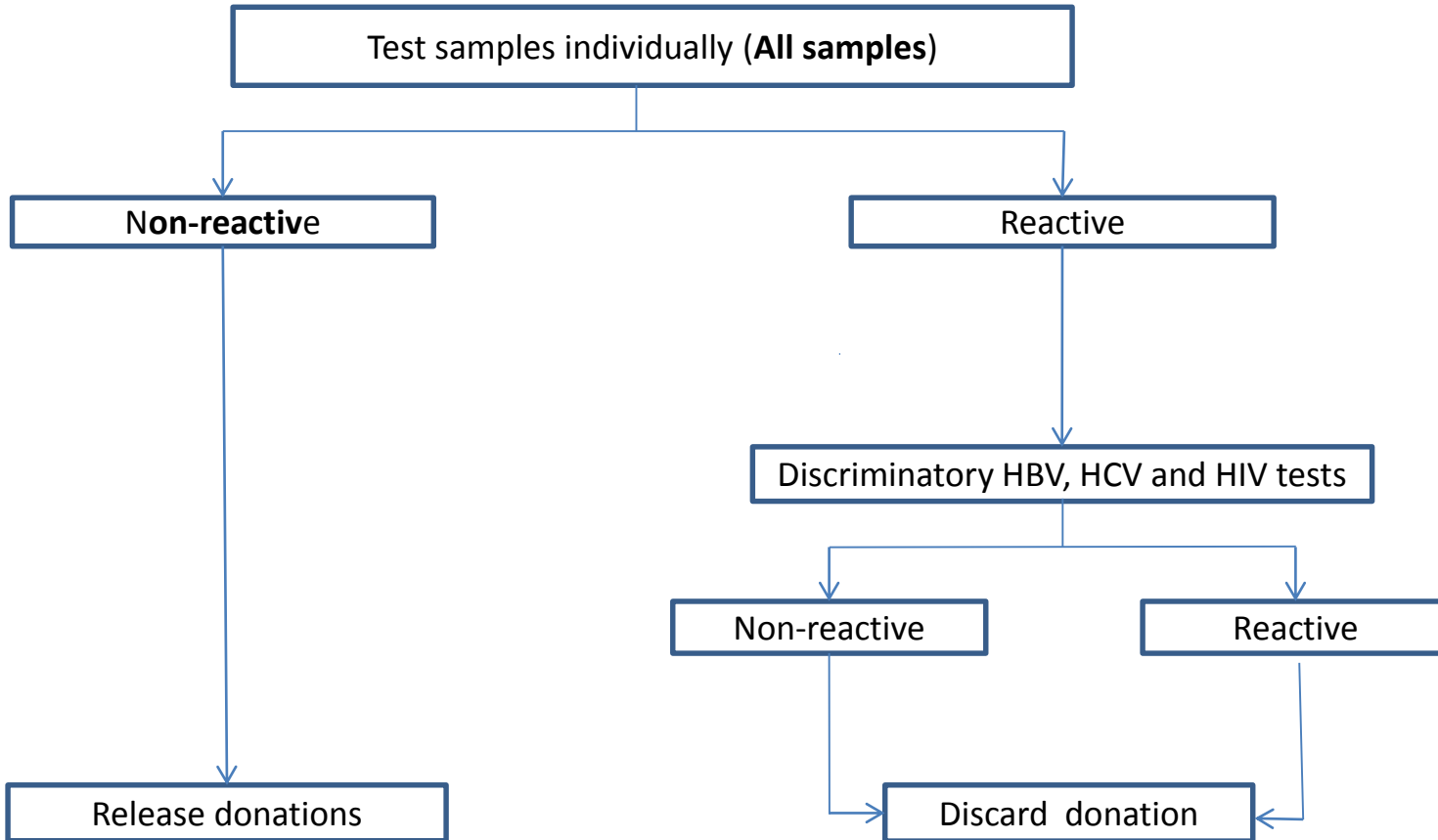
Background: Donor/Donation Risk Management

- Two basic algorithms for donor testing:
 - Mini-pool testing in mini-pools (MP) (Roche MP6: MPX, MPX v2 tests, Grifols MP8:UP)
 - Individual donor testing (IDT) (Grifols: Ultrio, Ultrio Plus, Ultrio Elite)
- No regulatory requirements for management of ID-NAT, non-repeat reactive (NRR) donors /donations
- MP6 NAT NRR donations transfused
- Algorithms used in different countries with different prevalence rates reviewed

Mini Pool (MP) Testing Algorithm (Pools of 6)



Individual Donor (ID) Testing Algorithm



NAT Screening Algorithm

- Repeat testing
 - Required to discriminate between true and false reactive result
 - Instrumental in recognising low viral load (OBI) (Poisson distribution issue?)
- Discriminatory testing: what are the issues?
- Different algorithms for high and low prevalence regions?

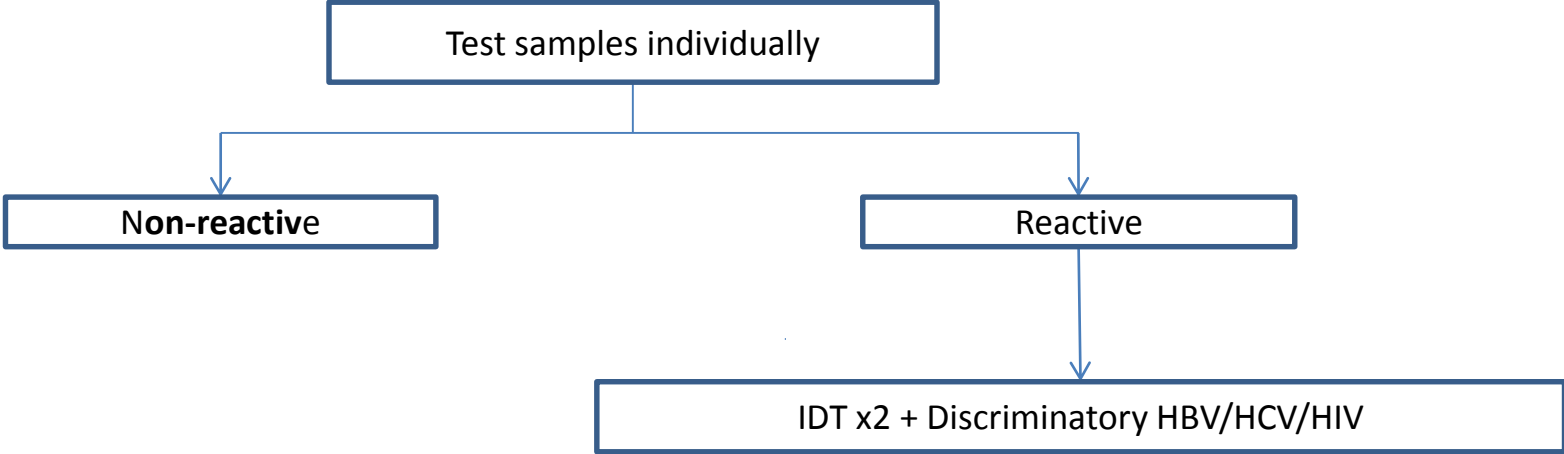
NAT Testing Algorithms

1. Joan O’Riordan (IBTS): IDT (Ultrio, Ultrio Plus, Ultrio Elite)
2. Marion Vermeulen (SANBS): IDT (Ultrio, Ultrio Plus)
3. Piotr Grabarczy (IHTM): IDT (Ultrio Plus, Ultrio Elite) + MP6 (MPX v2), MP8 (Ultrio Plus)
4. Lydia Blanco (CHH): IDT (Ultrio), MP6 (MPX, MPX v2)
5. Tasanee Sakuldamrongpanich(TRC): MP6 (MPX, MPX v2)

U, UP, UE: All donations (seronegative and seropositive) tested

MPX: Only seronegative donations tested

IDT NAT Algorithm (Ireland, S. Africa and Poland)



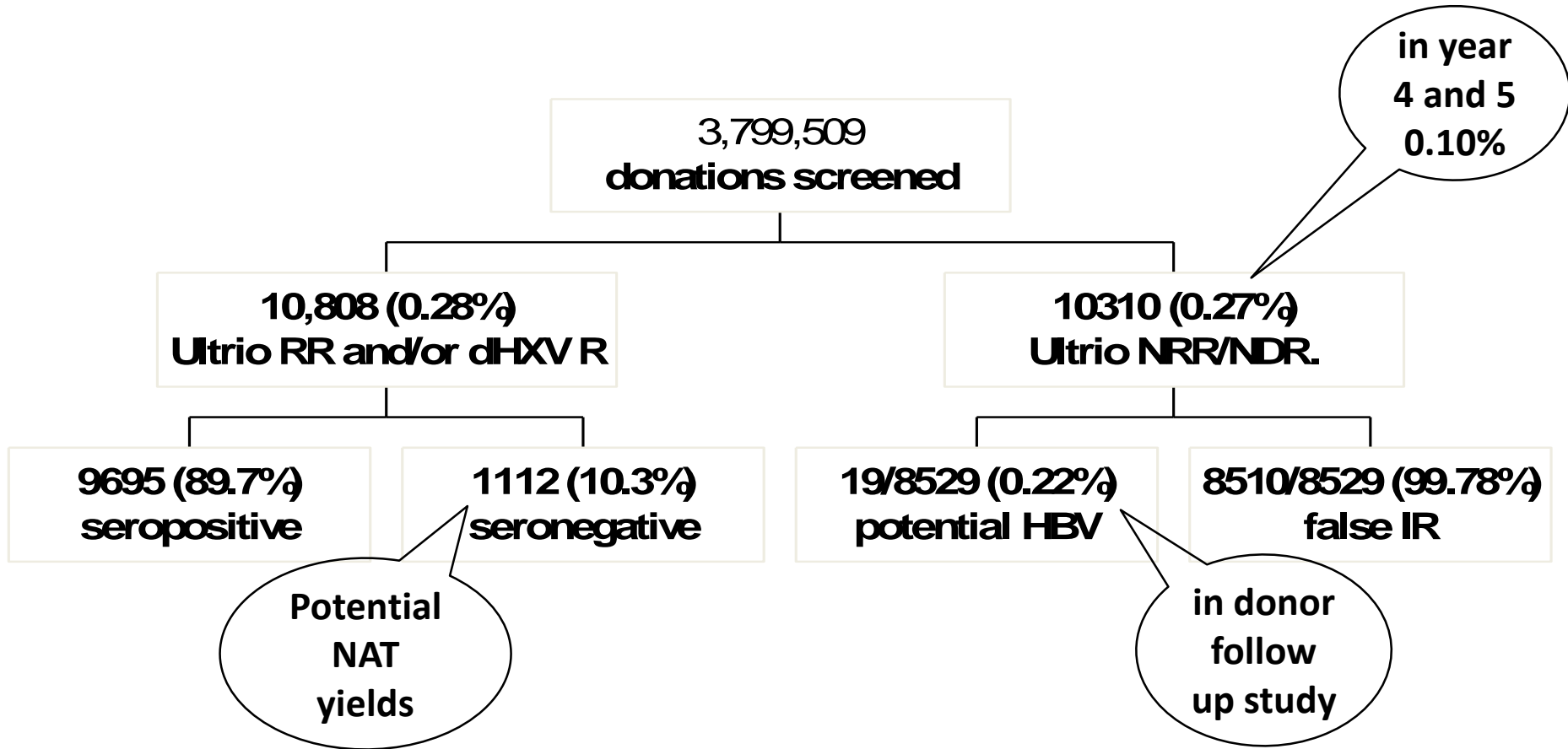
IBTS: Follow-up Studies of Ultrio Initial Reactive Donors

NRR/Discriminatory(+/-) confirmed by:

- Follow up
- Replicate Ultrio (2x), Discriminatory testing and serology

Tests/infection category (total no. tested)	Number (%)
IR	417
RR/Discriminatory (+)/Seropositive	31 (7.4%)
NRR & Discriminatory(+), all non-confirmed → false reactive	7 (1.7%)
NRR & anti-HBc → OBI	3 (0.4%)
NRR/Discriminatory(-)/Serology (-) → probably HBV WP	1 (0.2%)
NRR/Discriminatory (-)/Serology(-)	376 (90.2%)
NRR/Discriminatory(-)/Serology(-) confirmed or followed	369
NRR/Discriminatory(-)/Serology(-), all non-confirmed → false reactive	368 (368/369, 99.73%)*

South African National Blood Service (SANBS): Five Year ID-NAT Screening Data



SANBS: Confirmed NAT Yield Cases (S. Africa - HBV High Prevalence Region)

Confirmed by:

- Follow up
- Viral load on plasma unit
- Replicate Ultrio (5x) & Discriminatory HBV (5x) on plasma unit

NAT yield category	N (%)
Potential NAT yield (seronegative)	1,112 (100%)
• Confirmed positive	644 (57.9%)
• Unresolved	60 (5.3%)
• False reactive	408 (36.7%)

SANBS: Follow-up Studies of Ultrio Non-repeat, Reactive Donors

Follow-up sample
 NAT and serology (including anti-HBc)

Tests/infection category (total no. tested)	Number
NRR/Discriminatory(-)	10,310
NRR/Discriminatory(-)/Serology(-): Donors returning	8,529 (100%)
OBI infections in one of next bleeds	14 (0.164%)
HBV seroconversion in next bleed	5 (0.059%)
HIV seroconversion in next bleed	3 (0.035%)
False reactive	8,507 (99.74%)

IHTM: NAT Testing Algorithm (Poland - HBV Medium Prevalence Region)

IDT initial reactive/seronegative:

- 2 x Ultrio Plus/dHBV
- Confirmation at IHTM/HBV serology/follow for seroconversion or OBI

	IDT	MP6	MP8
Donations tested (pools tested)	305,141	450,750 (75,123)	244,831 (30,604)
IR (%)	186	62	12
Ultrio Plus NRR or MPX ID NR (%)	173 (93.0%)	49 (79%)	10 (83.3%)
Ultrio Plus RR or MPX ID R (%)	13 (7.5%)	13 (21%)	2 (16.5%)
HBV (OBI)	7	10	1
HCV	3	3	0
HIV	1	0	1
Unresolved (false reactive)	2		
% False reactive donations/pools	94.1%	79.0%	83.0%

CHH: NAT Testing Algorithm (Spain - HBV Medium/Low Prevalence Region)

Test (years)	Total no. pools tested	No. initial reactive pools	No. False reactive pools
MPX MP6 (06-11)	61,048	51	36 (70.6%)
MPX v2 MP6 (12)	21,648	20	18 (90%)

Pool reactive, individual donations non-reactive → initial reactive (false reactive pool)

Pool reactive, individual donation reactive, viral target not identified → repeat reactive, true positive pool and donation, unresolved

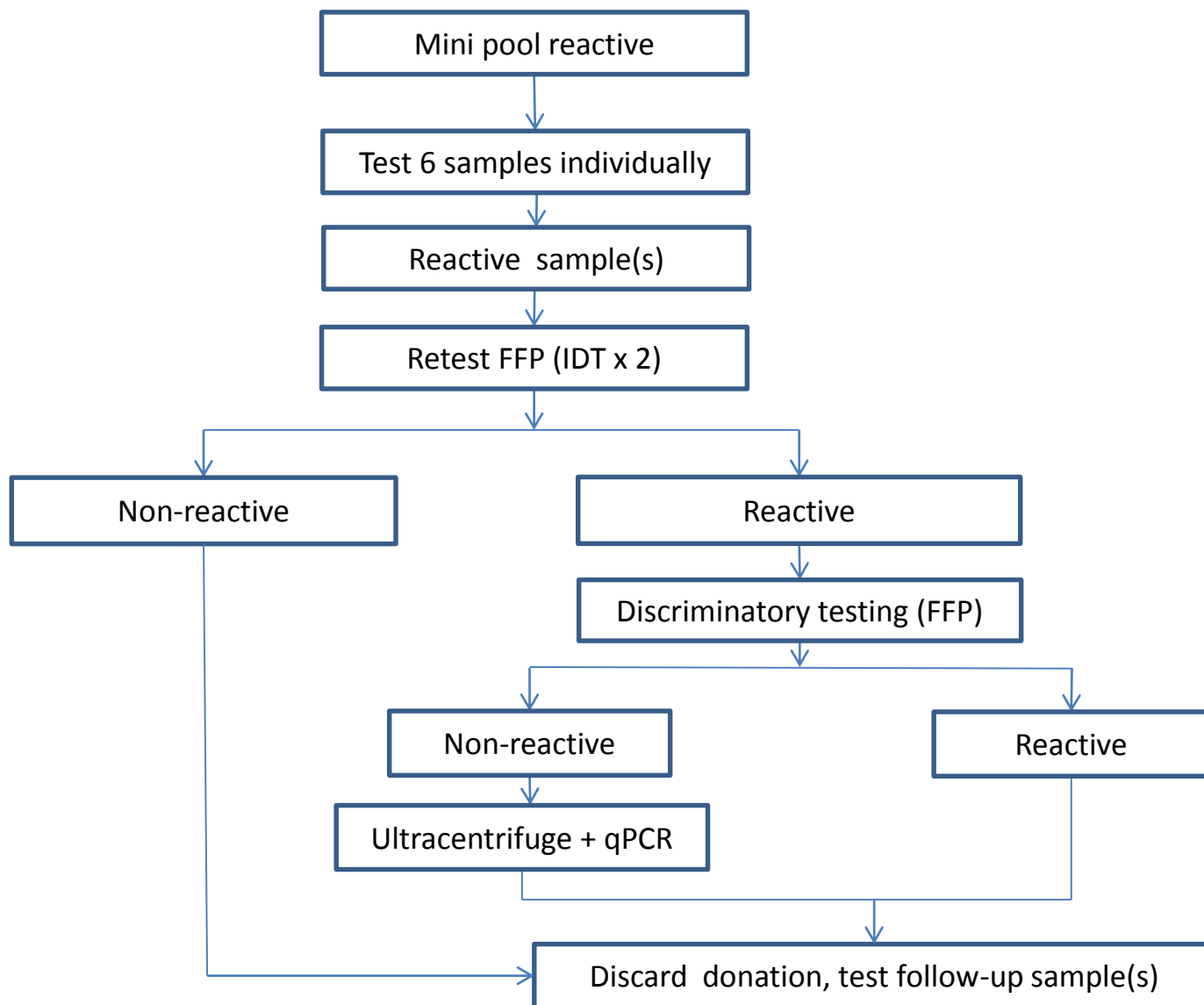
CHH: Pool R/IDT R/RXV NR

- 20011 – 18 samples MP6 R/IDT R/Discriminatory NR
- Follow samples tested by IDT, viral load and serology
 - All samples anti-HBc positive
 - 7/17 anti-HBs positive (3.3- 160 IU/mL)
 - 14/18 samples genotyped (10 genotype D. 4 genotype A2)
 - Viral load <10 IU/mL in 13 samples
 - 17/18 samples OBI (1 false reactive sample)
- Look-back and trace-back

CHH: Look-back of 13 OBI Donors

- Number of recipients – 158
- 45 died with no evidence of HBV infection
- Samples obtained from 23 recipients
 - 1 recipient HBV NAT positive/anti-HBc positive
 - 1 recipient anti-HBc positive (previous antibody status unknown)
 - 3 recipients anti-HBc/anti-HBs positive (previous antibody status unknown)
- 5/23 (22%) OBI? (background anti-HBc prevalence 15%)

TRC: Testing Algorithm for RR Samples

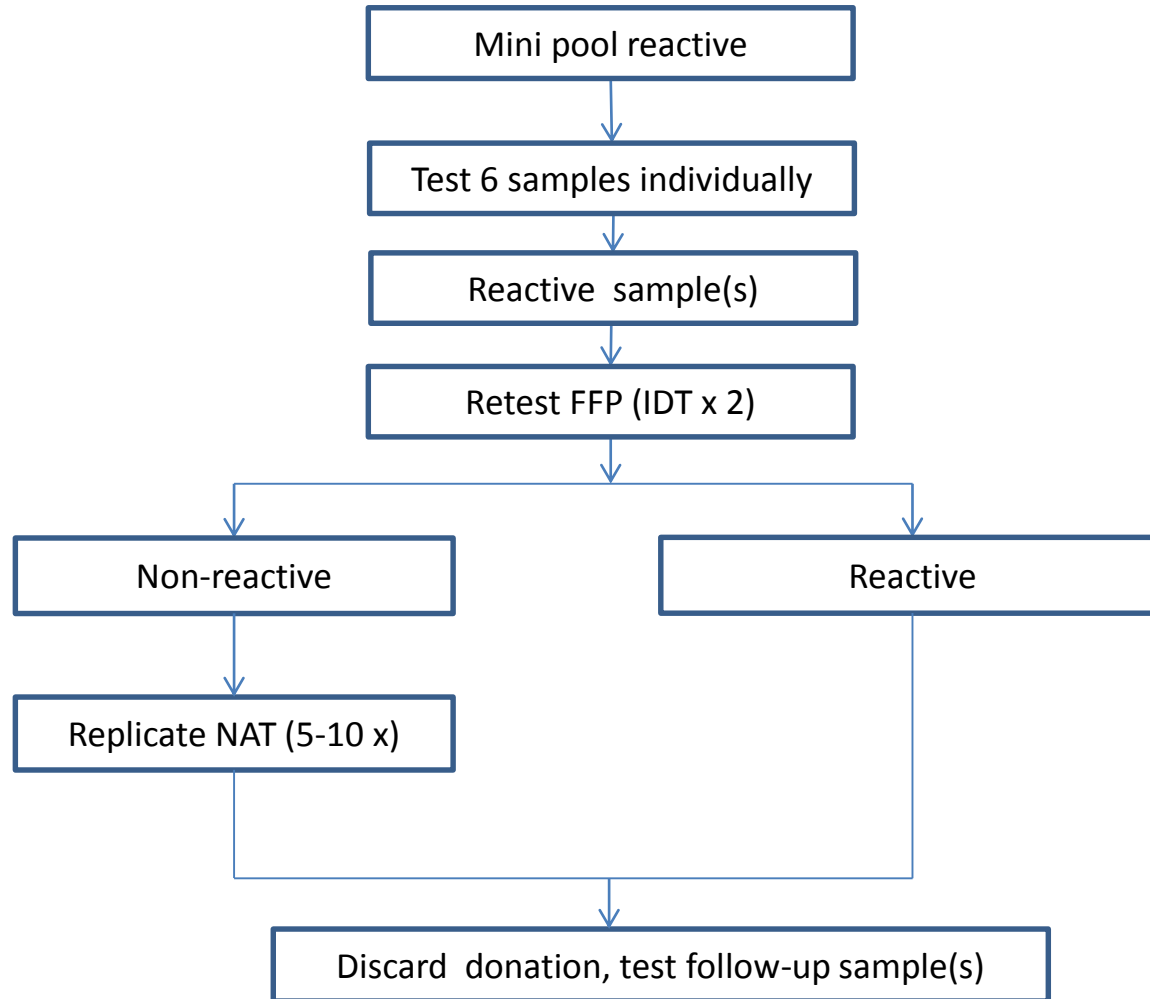


TRC: NAT Testing Algorithm (Thailand - HBV High Prevalence Region)

MPX testing , 2013

Results	Number (%)
Donations	595,059
Pools	99,180
IR Pool	437 (100%)
NRR (pool R/IDT NR)	189 (43.25%)
RR (pool R/IDT R)	248 (56.75%)
RR Donation	248 (100%)
RR donation/FFP NR	55 (22.2%)
31/55 RR donation/FFP NR follow-up samples tested by IDT and serology	
NAT R/ serology +	5 (16.1%)
Serology+	25 (80.6%)
False reactive (NAT NR/serology -)	1 (3.2%)

TRC: Testing Algorithm for RR Samples



TRC: NAT Testing Algorithm (Thailand - HBV High Prevalence Region)

MPX, v2 testing , 2014

Results	Number (%)
Donations	189,087
Pools	31,517
IR Pool	135 (100%)
NRR (pool R/IDT NR) Pool	87 (64.4%)
RR (pool R/IDT R) Pool	48 (35.6%)
RR Donation	48 (100%)
RR donation/FFP NR	4 (8.3%)
RR donation/FFP NR/replicate NAT + and/or sero +	3
False reactive donations	1 (2.1%)

Summary

IDT						
Site	Algorithm	Total donations tested	Initial Reactives NAT yield (%)	Initial Reactives Confirmatory testing or Followed	Reactive	False reactive (%)
Ireland	IDT (UP)	743,453	386 (0.052%)	379	4 (3 anti-HBc, 1 HBV DNA+ with TaqMan)	375 (92.14%)
S. Africa	IDT (UP)	3,799,409	11,422 (0.301%)	9,641	427	8,915 (92.47%)
Poland	IDT (UP)	305,141	186 (0.061%)	186	11	175 (94.09%)
MPX						
Site	Algorithm	Total donations tested	Initial Reactive pool NAT yield (%)	Initial Reactive Samples confirmatory testing or followed	Reactive	False reactive (%)
Poland	MP6 (MPX)	75,123	62 (0.083%)	62	13	49 (79.03%)
Poland	MP8 (UP)	30,604	12 (0.039%)	12	2	10 (83.33%)
Spain	MP6 (MPX)	61,048	87 (0.143%)		51	36 (70.59%)
	MP6 (MPX v2)	21,648	20 (0.092%)	20	2	18 (90.00%)
Thailand	MP6 (MPX)	99,180	437 (0.441%)	413	198	215 (52.06%)
	MP6 (MPX v2)	31,517	135 (0.428%)	135	44	91 (67.41%)

Summary

- Small incremental risk if ID-NAT non-repeat reactive units transfused
- This risk only small fraction of overall residual risk
- Different algorithms for high and low prevalence regions
- Samples with low viral loads, usually OBI give non-repeat reactive NAT results and/or Discriminatory non-reactive results

- Repeat testing required to identify low positive/false reactive samples
- Pool: MP followed by IDT
- IDT: followed by IDT x2 (at least)
- Issues of contamination: NAT testing of all samples, especially in regions of high prevalence
- Specificity of NAT assay
- Relative sensitivities of NAT assay and discriminatory assays

Summary

- Current algorithms for MP and IDT (package inserts and Regulatory approved)
- MP: Pool followed by IDT → false reactives and allows these to be released
- IDT: IDT followed by Discriminatory testing does not, by itself, detect false reactives and additional testing required
- High prevalence regions require additional testing (FFP, follow-up samples, HBV serology, look-back, trace-back) to eliminate false reactives
- Other issues may be more important for TTD – viral variants (e.g. HIV-1)

CHH: Pool R/IDT R/RXV NR (MPX, 2011)

Donors	ID PCR	Anti-HBc	HBs Ag	Anti-HBs
1	+	+	-	+
2	+	+	-	160.0 UI/L
3	+	+	-	-
4	+	+	-	-
5	+	+	-	-
6	+	+	-	-
7	+	+	-	?
8	+	+	-	-
9	+	+	-	-
10	+	+	-	-
11	+	+	-	39.1 IU/L
12	+	+	-	3.3 IU/L
13	+	+	-	39.7 IU/L
14	+	+	-	44.13 IU/L
15	+	+	-	43.04 IU/L
16	+	+	-	-
17	+	+	-	-
18	+	+	-	58.90 IU/L

CHH: Trace-back of 18 Donors

Donor	No. previous donations	Anti-HBc previous donations	HBV PCR previous donations
1	40	+	NA
2	5	+	NA
3	4	+	NEGATIVE
4	61	+	NA
5	4	+	NA
6	5	+	NEGATIVE
7	9	+	NEGATIVE
8	2	+	NEGATIVE
9	6	+	NEGATIVE
10	4	+	NEGATIVE
11	4	+	NEGATIVE
12	3	NEGATIVE	NEGATIVE
13	2	+	NA
14	1	+	NEGATIVE
15	12	NA	NEGATIVE
16	11	NA	NEGATIVE
17	8	NA	NEGATIVE
18	1	NA	NEGATIVE