The problems with D genotyping

Joint UK NEQAS BTLP / BBTS SIG

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NHS Blood and Transplant

Objectives

- Empower you to make informed decisions relating to *RHD* genotyping
- Know the purpose of each test
- Interaction
  - Please find your coloured paper
- Have a giggle!
- Explain *RHD* genotyping
  - Basics
  - Barriers
  - “standard *RH* genotype”
  - “Weak D”

What is that on Shane's face?

He's growing it for Movember

It's a lifestyle choice

Homage for Freddie Mercury

It's the hair he lost from his forehead

What are the problems with D genotyping?

It’s too confusing

I have to refer samples

Nothing, it’s perfect

It can give you the wrong answer

What are the problems with D genotyping?

It can give you the wrong answer

Blood Group Genotyping by Molecular Genetics

No suitable red cell sample

Transfused patients

DAT positive

Fetal typing

More / better information

No serology reagent available

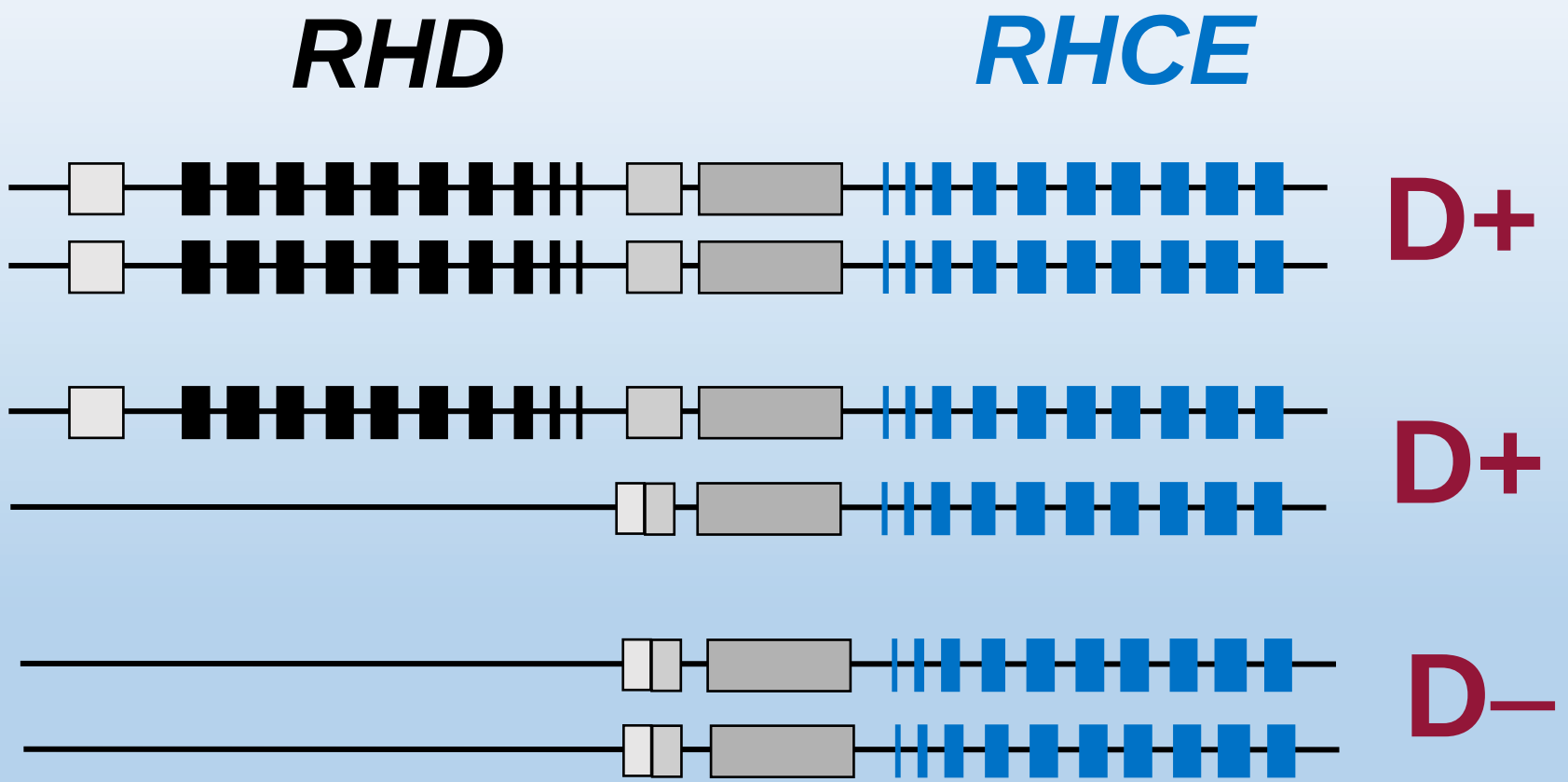
Identify specific variants

Zygosity testing

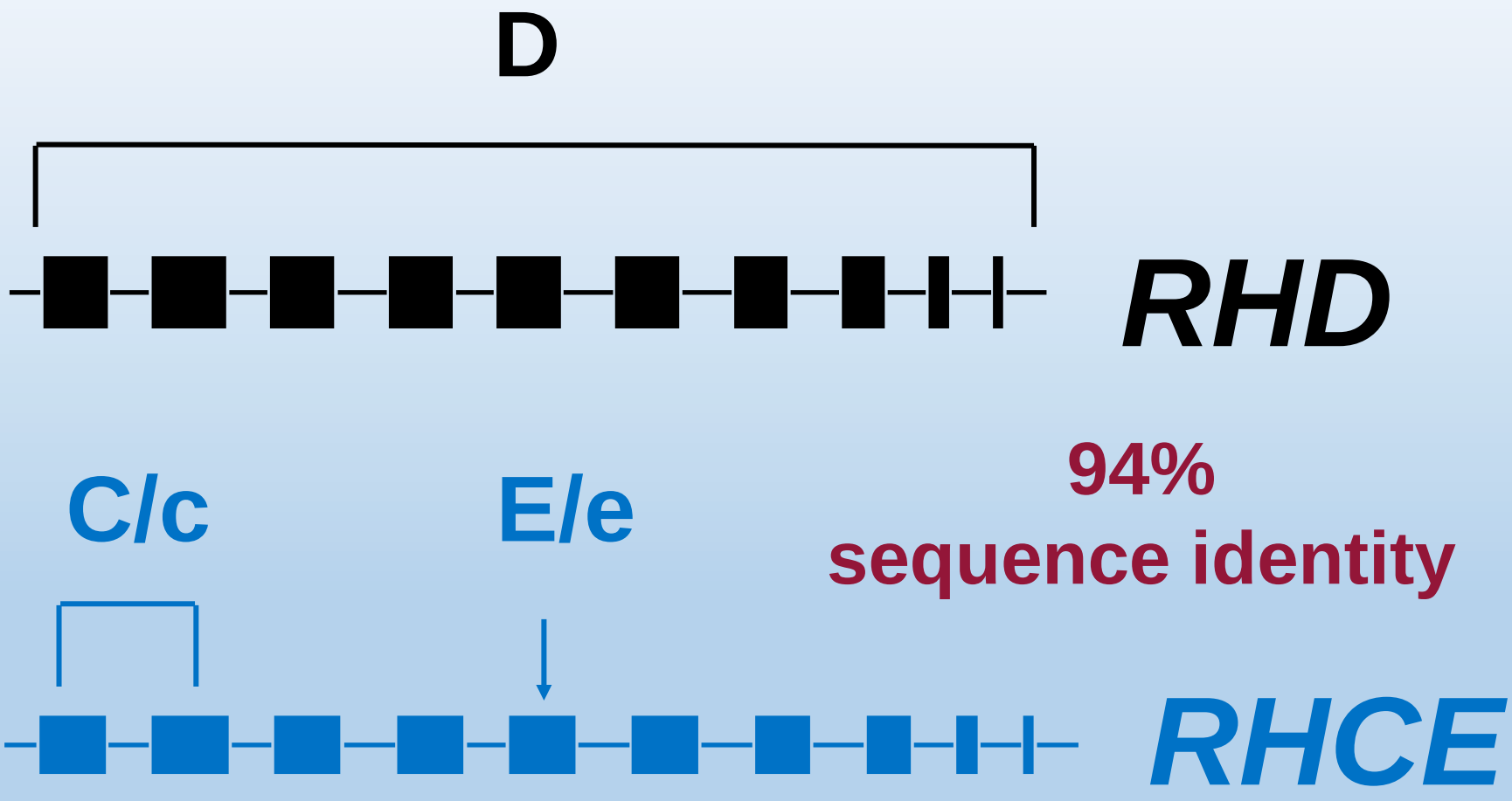
More efficient / cost effective

https://ashpublications.org/bloodadvances/article/4/15/3495/461690/Development-and-validation-of-a-universal-blood …donor genotyping platform

RHD Basics



RHD Basics



RHD Basics

RHD AGAAGTGGTTTCAGGATCAGCAAAGCAGGGAGGATGTTACAGGGTTGCCTTGTTCCCAGC

RHCE AGAGGTGGTTTCAGGATCAGCAAAGCAGGGAGGATGTTACAGGGTTGCCTTGTTCCCAGC

RHD Basics

```
RHD     AGAAGTGGTTTCAGGATCAGCAAAGCAGGGAGGATGTTACAGGGTTGCCTTGTTCCCAGC
RHCE    AGAGGTGGTTTCAGGATCAGCAAAGCAGGGAGGATGTTACAGGGTTGCCTTGTTCCCAGC
        ***  **********************************************************
```

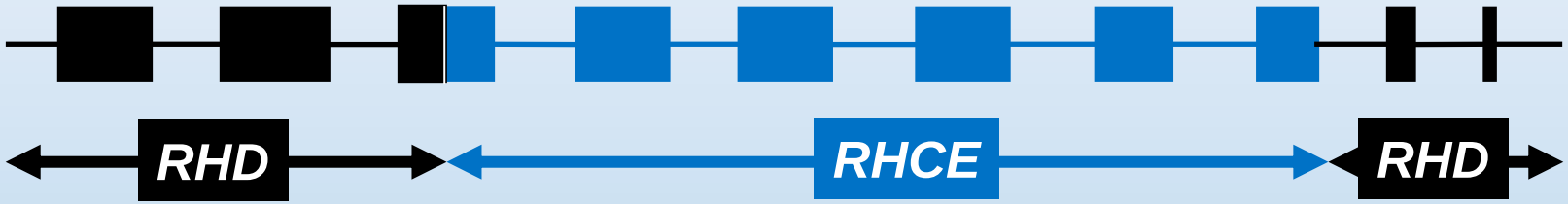
Must target differences between *RHD* and *RHCE*

RHD Barriers

Only 18% of
D- Black people
are homozygous
for a deletion of *RHD*

RHD Barriers

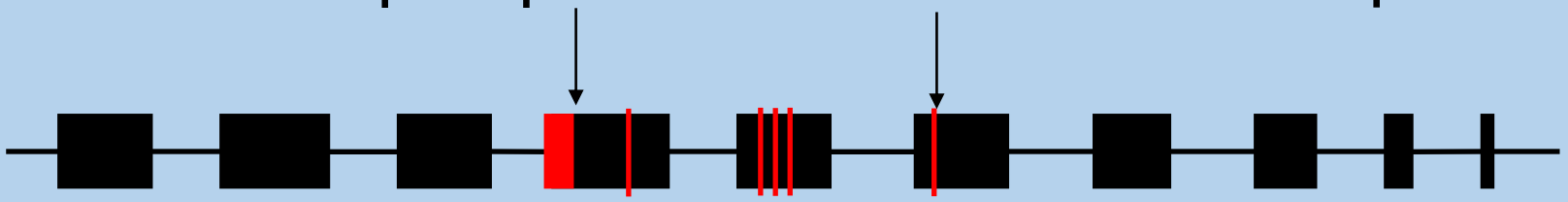
15% have *RHD-CE-D^s*



D-

66% have *RHDΨ*

37 bp duplication nonsense → stop



D-

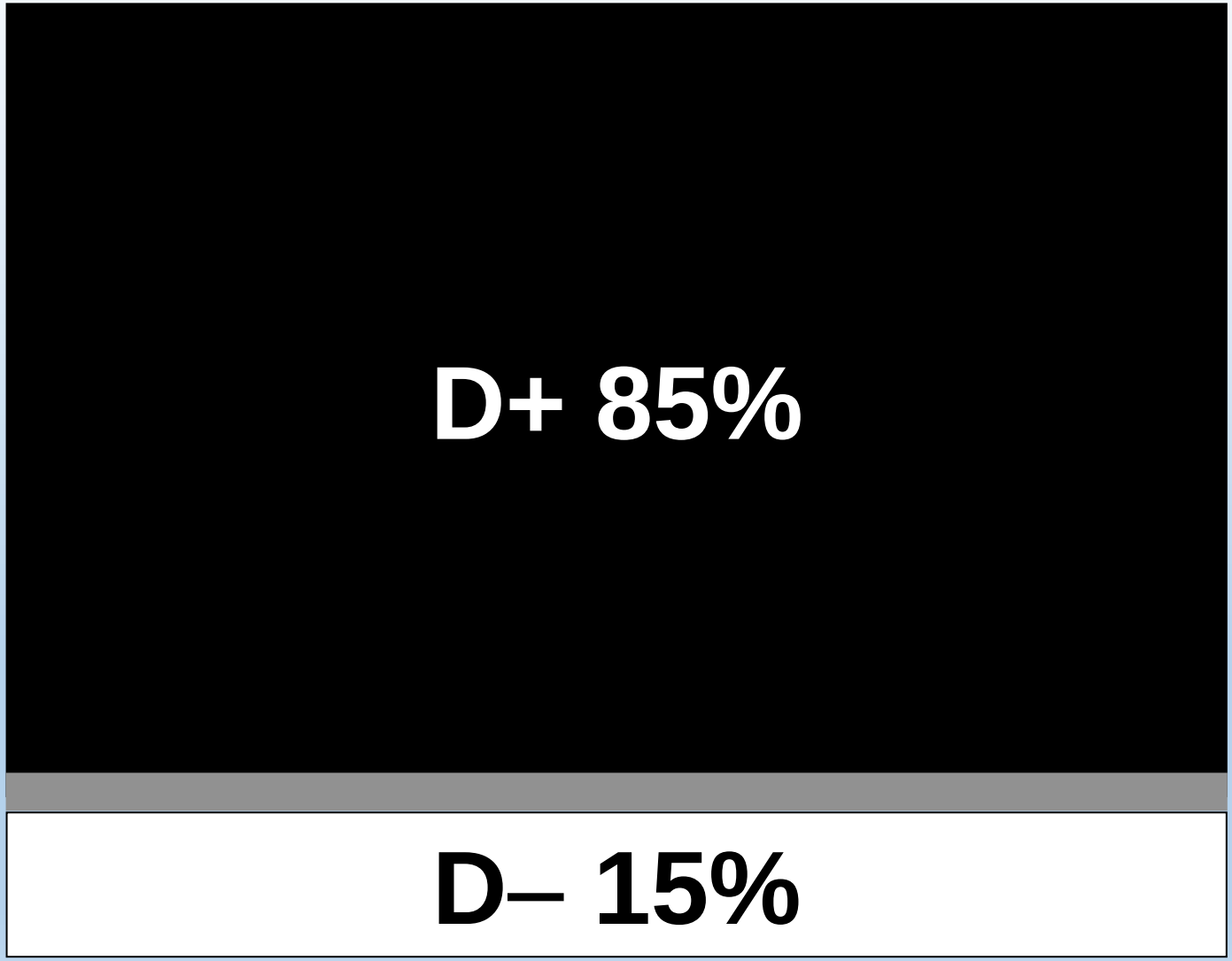
RHD Barriers

Must return result of D- for *RHD-CE-D*^{s} and *RHD*Ψ*

Standard *RH* Genotype

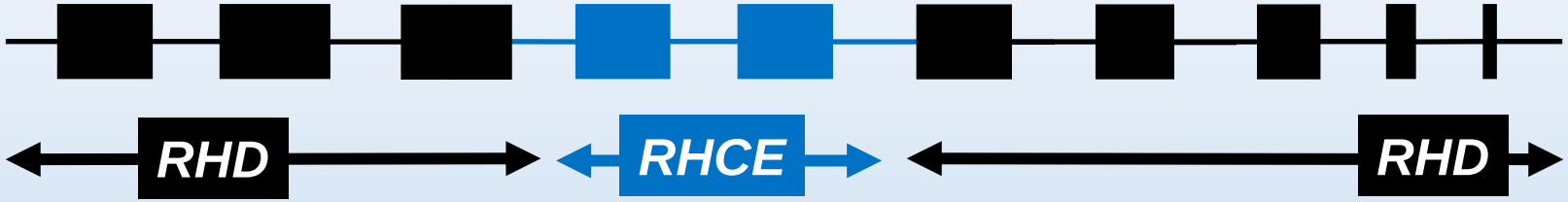
- Exon 4
  - If RHD*Ψ is present → detection of PCR product 37bp bigger = D-
- Exon 7
  - If *RHD*D-CE-D^{s}* only is present → no amplification of Exon 4 and 7 = D-

RHD Barriers



<1% variant

RHD Barriers



DVI

- can produce anti-D as a patient
- can immunize D- recipients
- lack exons 4 and 5 of *RHD*

Must return result of D- for DVI (PATIENT)

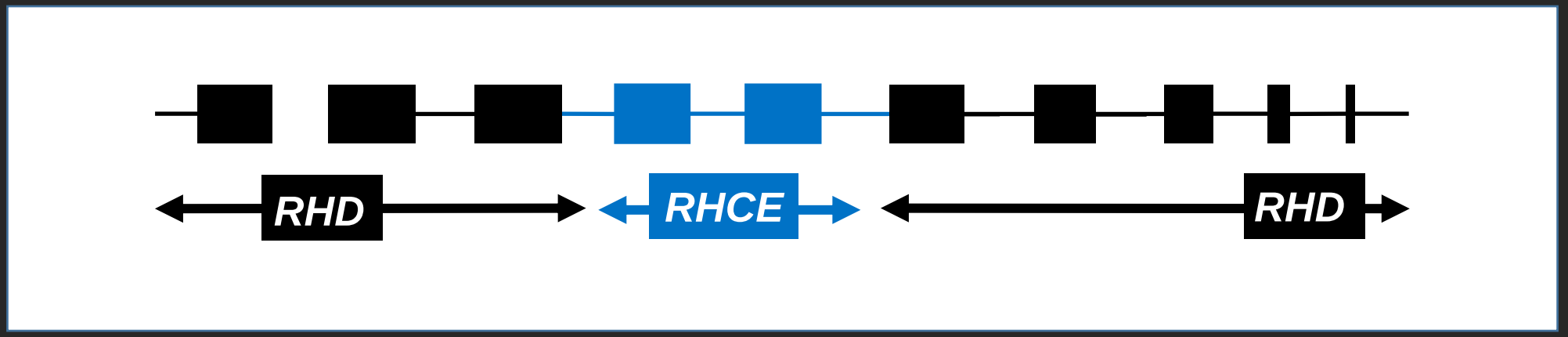
What results would standard *RH* genotype give for DVI?

Exon 4+ 7+

Exon 4- 7-

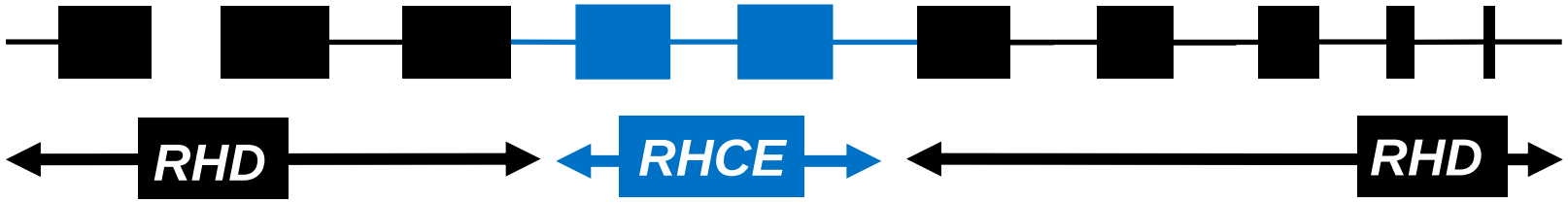
Exon 4+ 7-

Exon 4- 7+



What results would standard *RH* genotype give for DVI?

Exon 4- 7+

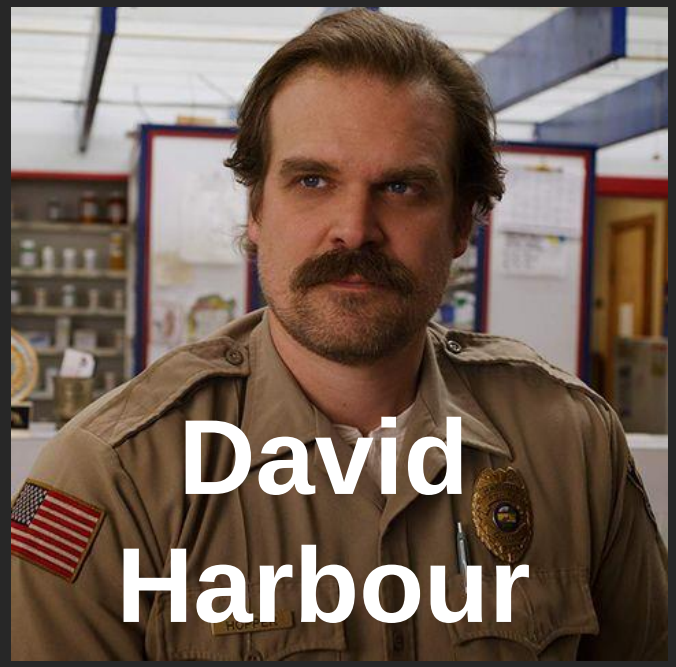


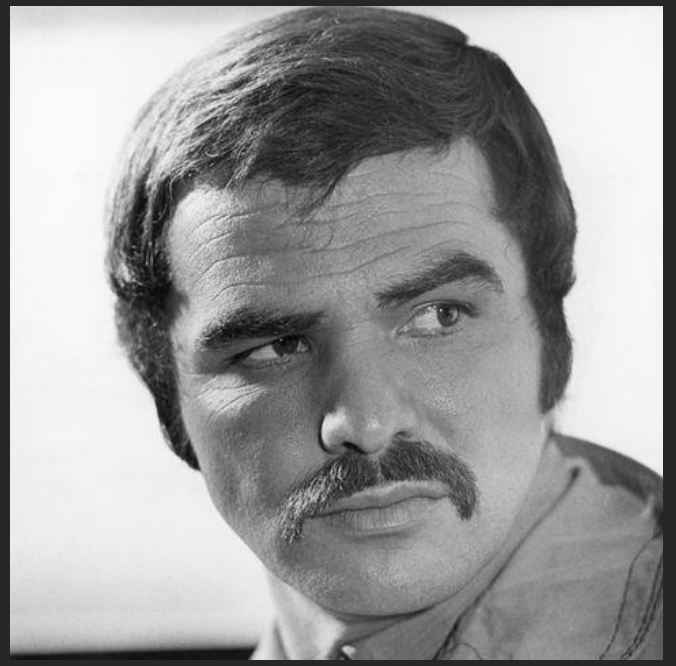
Standard *RH* Genotype

- Common variants in UK population include:
  - *RHDΨ*, D-
  - *RHD-CE-D*^{S} , D-
  - *RHDVI*, D+^{var}, treat as D-

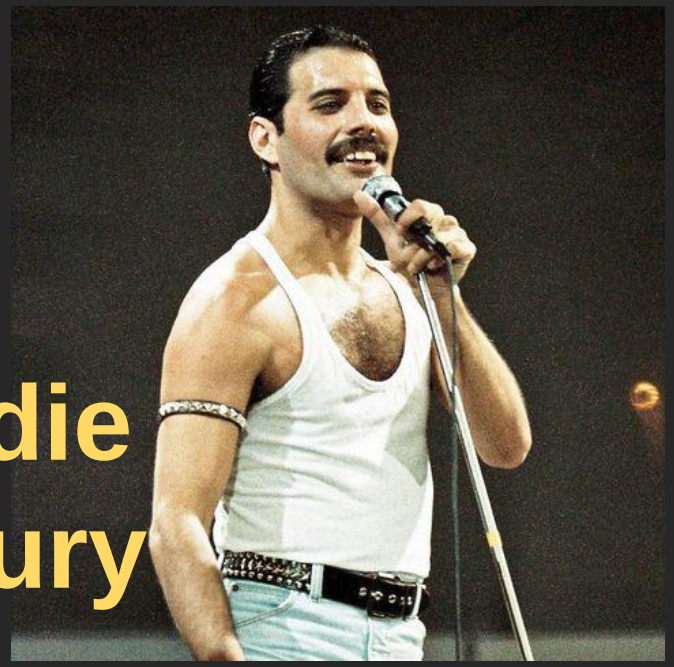
Standard *RH* genotype is accurate

Shane's moustache looks most like?





Burt Reynolds



Frida Kahlo

Standard *RH* Genotype

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…for most patients

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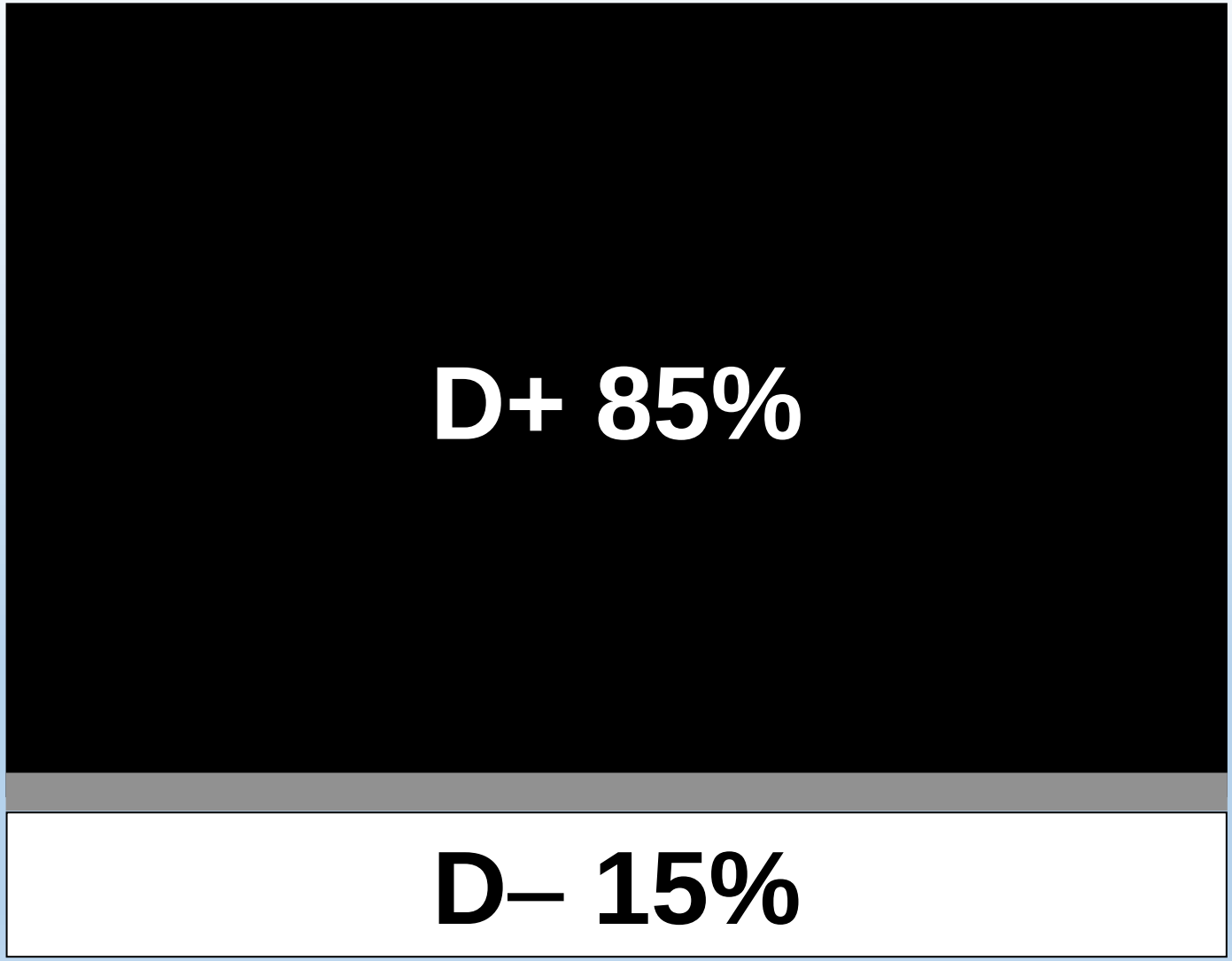
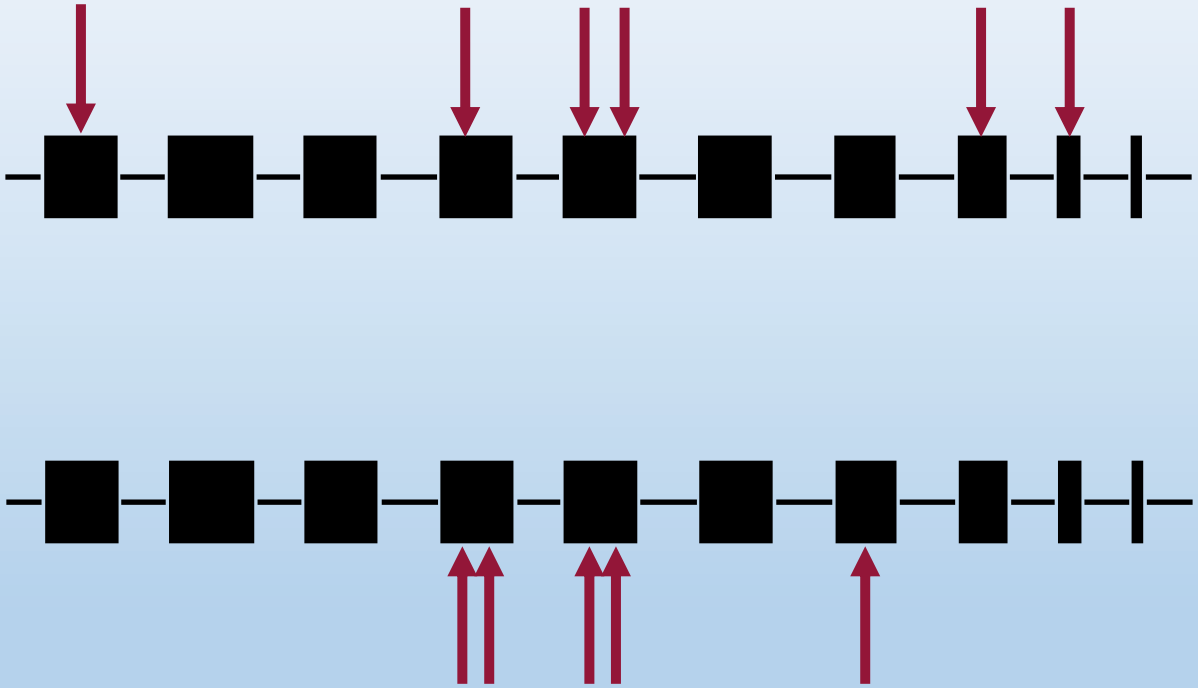


<1% variant

Standard *RH* Genotype

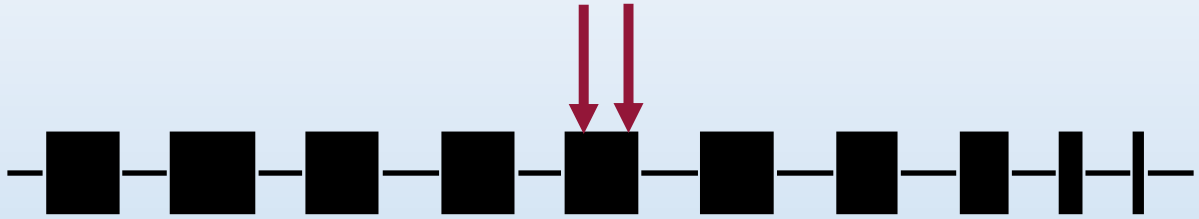


Hundreds of *RHD* variants

Produce anti-D

e.g. DV

Standard *RH* Genotype



Hundreds of *RHD* variants

Produce anti-D

e.g. DV

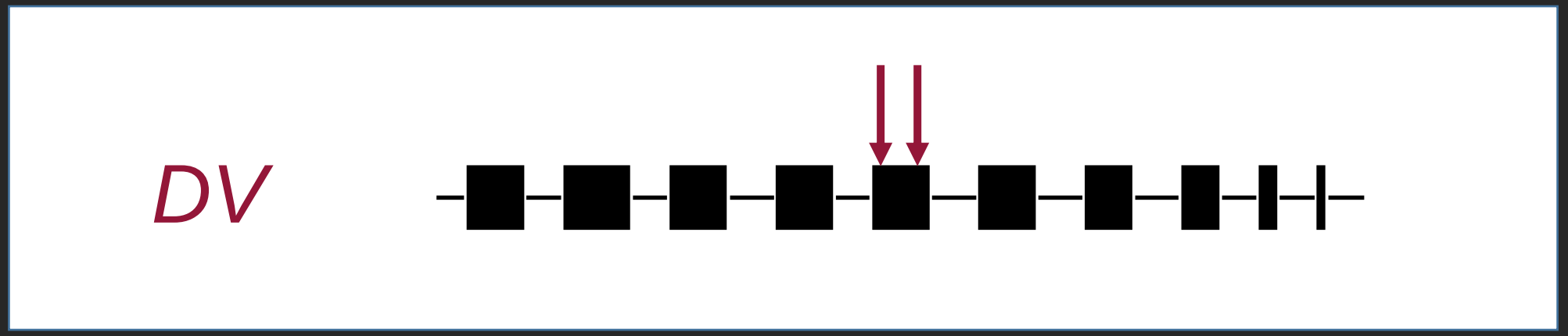
What results would standard *RH* genotype give for DV?

Exon 4+ 7+

Exon 4- 7-

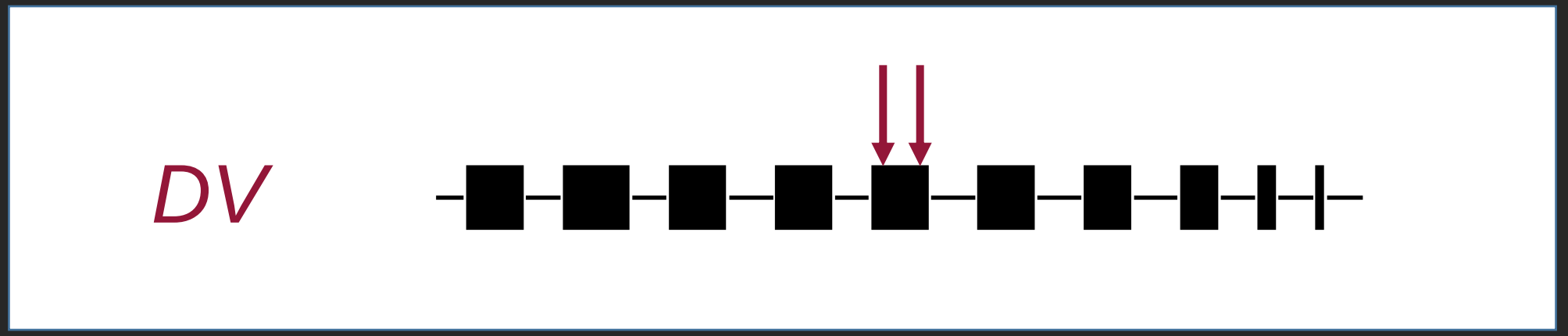
Exon 4+ 7-

Exon 4- 7+



Exon 4+ 7+

What results would standard *RH* genotype give for DV?



What are the problems with D genotyping?

It’s too confusing

I have to refer samples

Nothing, it’s perfect

It can give you the wrong answer

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Standard *RH* genotype is accurate

… for most patients

NOT if you suspect a *RHD* variant e.g. anti-D, D+^{var}

Weak D

Express all epitopes of D, can safely be treated as D+

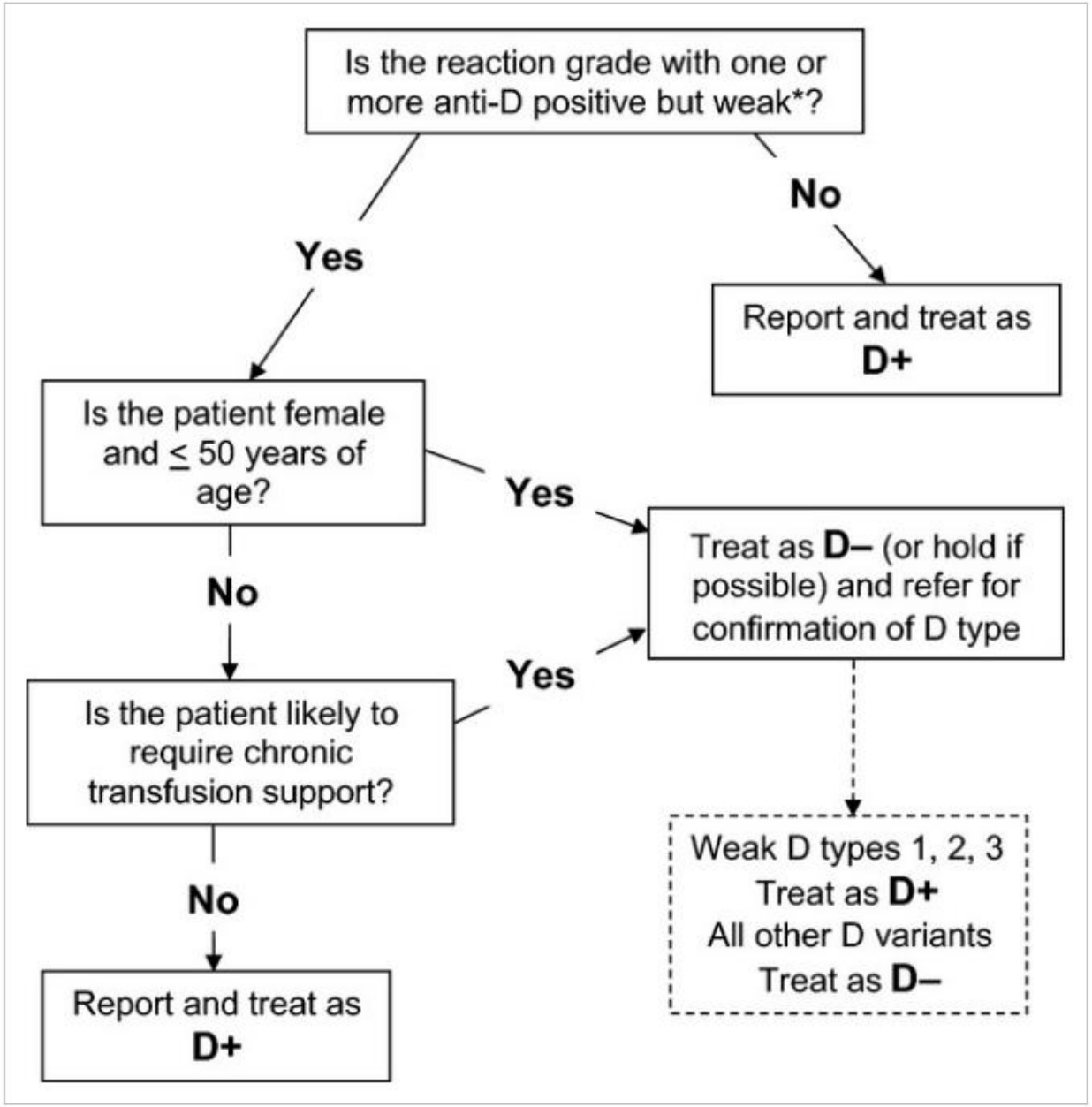
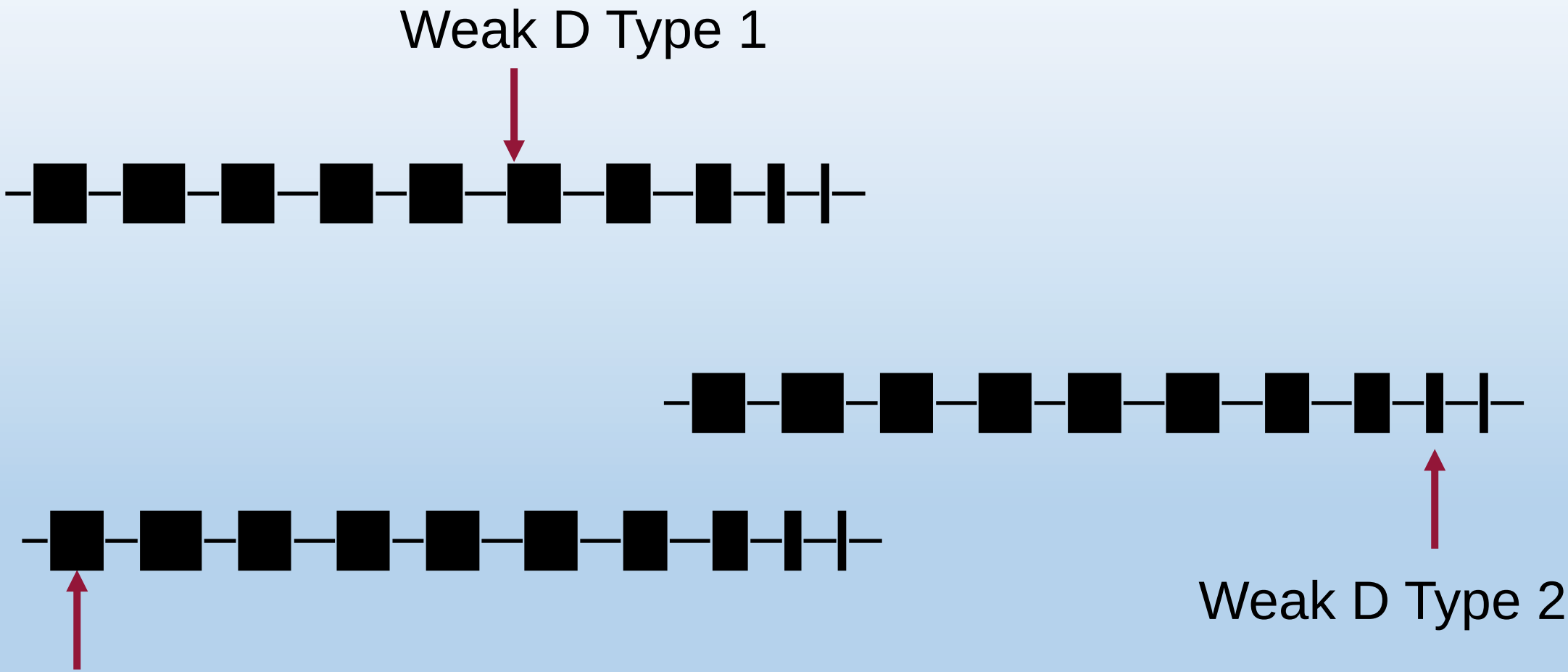


Figure 3 Open in figure viewer | PowerPoint

Reporting of D typing anomalies and selection of red cells. Adapted from: (Milkins *et al*, 2013)

Weak D



Weak D

If SNP responsible for Weak D Type 1, 2 or 3 is present
→ e.g. Weak D Type 1

What results would Weak D genotype give for Weak D Type 1?

Weak D Type 2

Weak D Type 1, 2, 3 negative

Weak D Type 1

What results would Weak D genotype give for Weak D Type 1?

Weak D Type 1

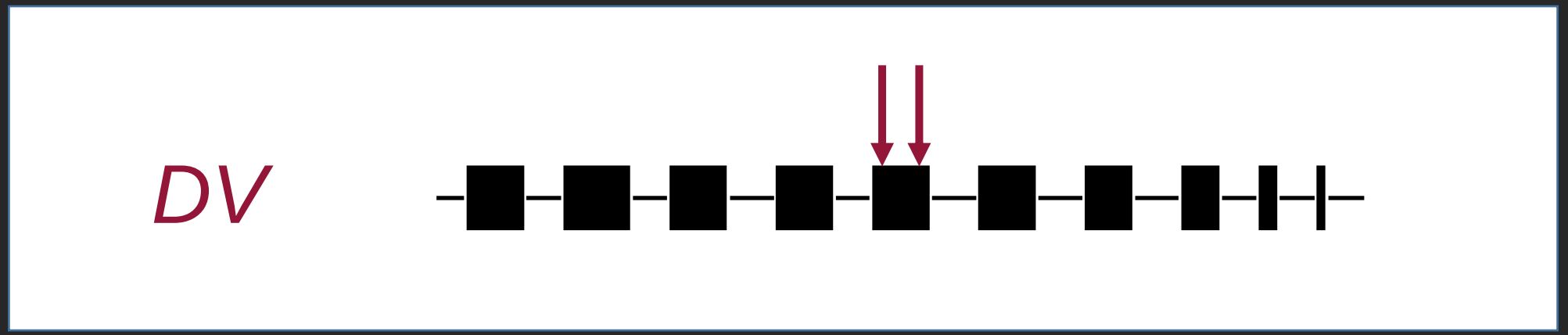
Would you treat this patient as D+ or D-?

What results would Weak D genotype give for DV?

Weak D Type 2

Weak D Type 1, 2, 3 negative

Weak D Type 1



What results would Weak D genotype give for DV?

Weak D Type 1, 2, 3 negative

Would you treat this patient as D+ or D-?

The problems with D Genotyping

No evidence of *RH* variant → Standard *RH* genotype

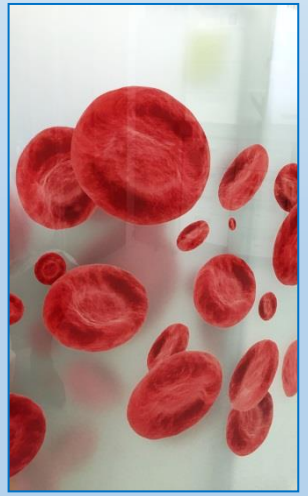
Evidence of *RH* variant → Weak D genotype

Unsure → explanation on referral form, call

Red Cell Genotype tests and indications

Name	General scope / indications	Outline of *RH* targets	Other blood groups	Sample / Lab	document	TrT
Standard Genotype	Common blood group antigens with no evidence / suspicion of variants	Presence or absence of *RHD*. SNP for C, c, E, e	K/k, $Fy^{a/b}$, $Jk^{a/b}$, M/N, S/s, U-, $U+^{var}$	3ml EDTA / MD	FRM4738 INF1341	10 days (48 hrs for Urgent)
BEADchip	Common and extended blood group antigens with no evidence / suspicion of variants	Presence or absence of *RHD*. SNP for C, c, E. Some e variant alleles including V, VS	K/k, $Kp^{a/b}$, $Js^{a/b}$, $Fy^{a/b}$, Fy^{X}, $Jk^{a/b}$, M/N, S/s, U-, $U+^{var}$, $Lu^{a/b}$, $Di^{a/b}$, $Co^{a/b}$, $Do^{a/b}$, $LW^{a/b}$, Sc	3ml EDTA / MD	FRM4738 INF1341	10 days
HGP	Common blood group antigens and more common *RHD, RHCE* and *GYPB* variants. Useful for Tx dependent Black patients and suspicion of anti-e when e+	Detection of *D, C* and *e* variant alleles more common in Blacks and likely to appear D+, C+ or e+ on routine phenotype. Includes V / VS and hr^{S} / hr^{B}	K/k, $Kp^{a/b}$, $Js^{a/b}$,$Do^{a/b}$, $Fy^{a/b}$, $Jk^{a/b}$, M/N, S/s, U-, $U+^{var}$	3ml EDTA / MD	FRM1597	12 weeks
Weak D 1,2	In the presence of an aberrant D phenotype. To guide treatment as D+ or D-	Presence or absence of *RHD*Weak D Type 1* and *2* alleles <u>only</u>	N/A	3ml EDTA / RCR	via RCI only	6 weeks
RCI genotype	Common blood group antigens with no evidence / suspicion of variants At digression of RCI / urgent cases.	*RHD* NOT reported. SNP for C, c, E, e	K/k, $Fy^{a/b}$, $Jk^{a/b}$, M/N, S/s, U-, $U+^{var}$ $Do^{a/b}$	3ml EDTA / RCI	1A	3 hours

Thank You



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