Performance and variability of Factor XIII Screening: Data from UK NEQAS Surveys

F E Preston

Factor XIII

- Deficiency leads to characteristic bleeding history
- 1% factor XIII sufficient for haemostasis
- Simple clot solubility test sensitive to ~1% factor XIII in plasma

Factor XIII Deficiency

- A rare autosomal recessive disorder
- Affects 1 in 1-3 million individuals
- Most common bleeding manifestations
  - Subcutaneous bleeding (57%)
  - Delayed umbilical cord bleeding (56%)
  - Intramuscular bleeds (49%)
  - Postoperative haemorrhage (40%)
  - Haemarthroses (36%)
  - Intracerebral haemorrhage (34%)

Factor XIII
Diagnosis

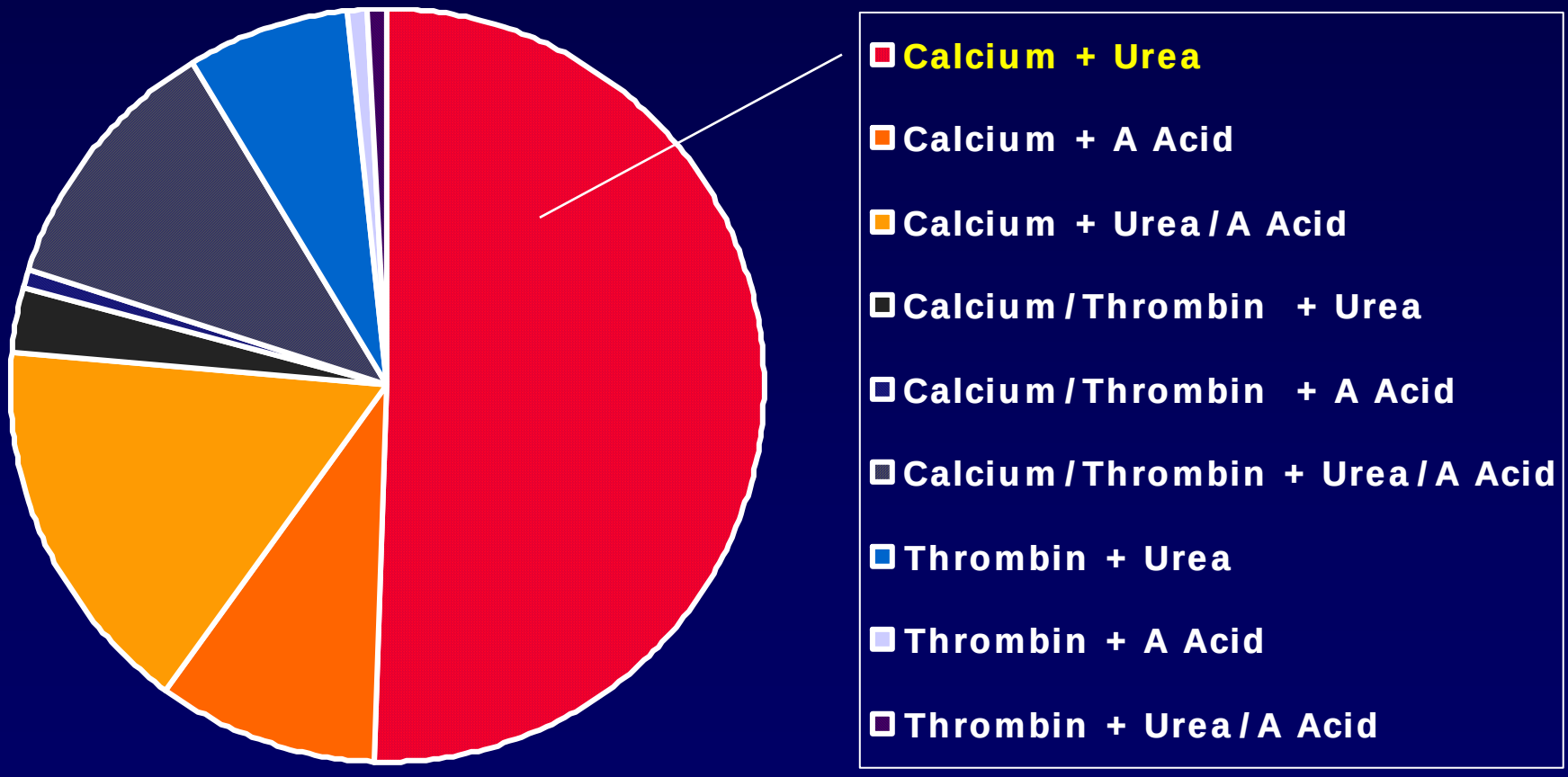
- Conventional coagulation tests normal
- Screen test - Clot solubility test
  - Based on solubility of non-crosslinked fibrin in 5mol/L urea, 1% monochloroacetic acid or 2% acetic acid
  - Plasma clotted with M/40 calcium chloride
  - Dissolution of the clot indicates low levels of FXIII:C

Factor XIII
UK NEQAS surveys 1998/99

Sample type	FXIII assay median (u/dl)	Interpretation (%)		
		Normal	Borderline	Abnormal
Normal	108.0	98	0	2
FXIII deficient pool	6.7	3	2	95
Factor XIII deficiency - trough level	8.0	70	5	25

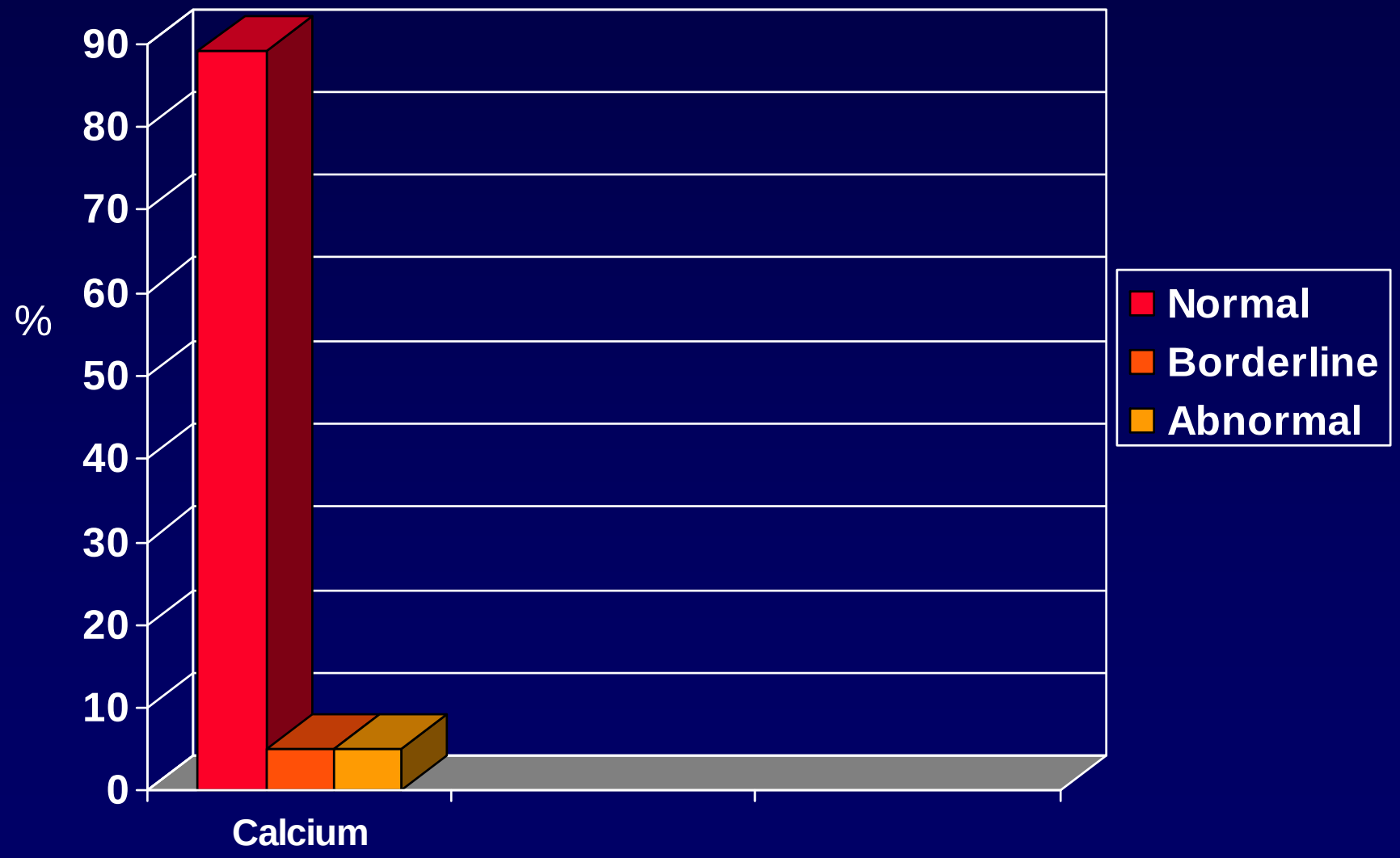
UK NEQAS : Methods in use

Factor XIII screening exercise, May 1997



Factor XIII approx. 8u/dl

Interpretations by clotting agent



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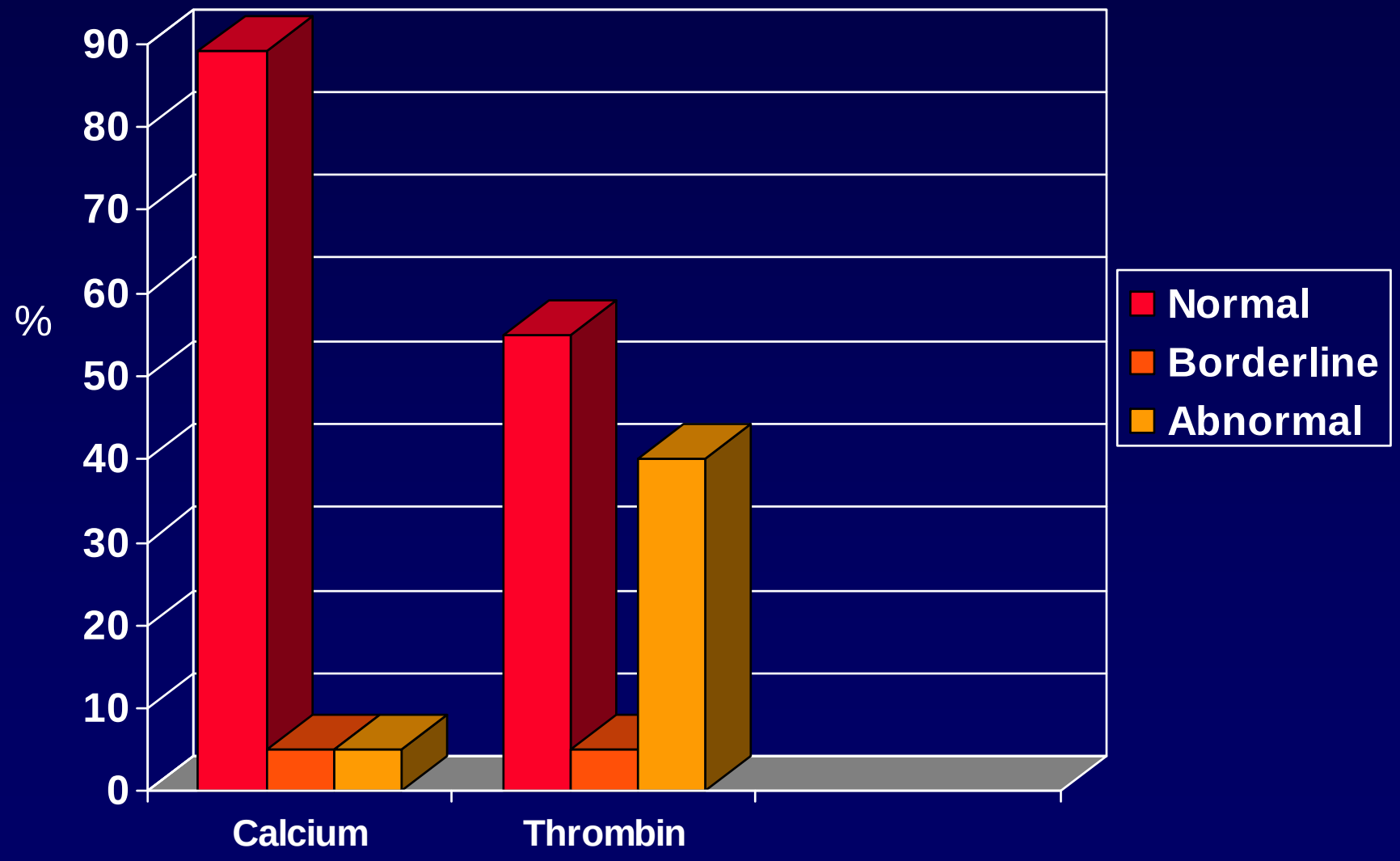

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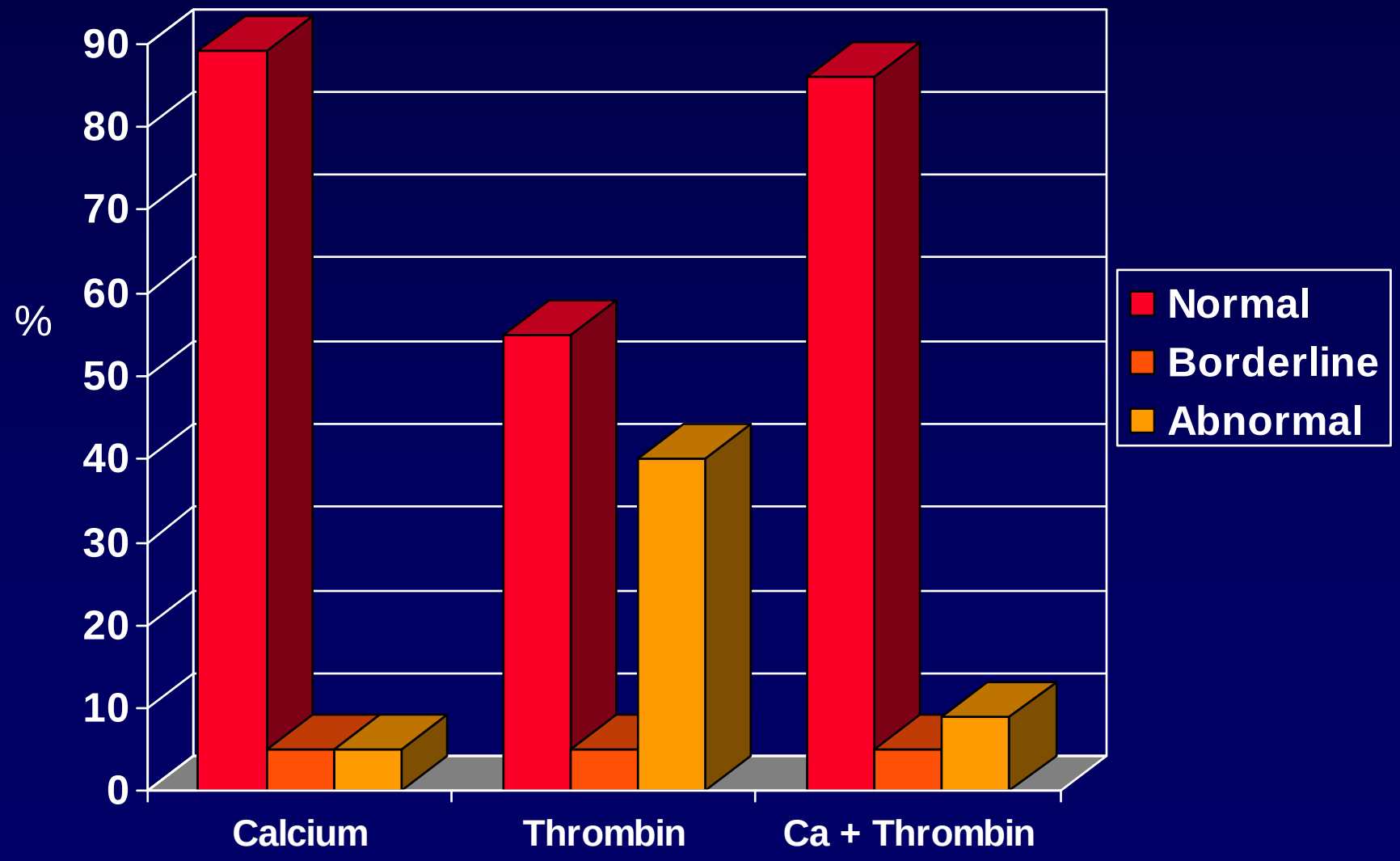
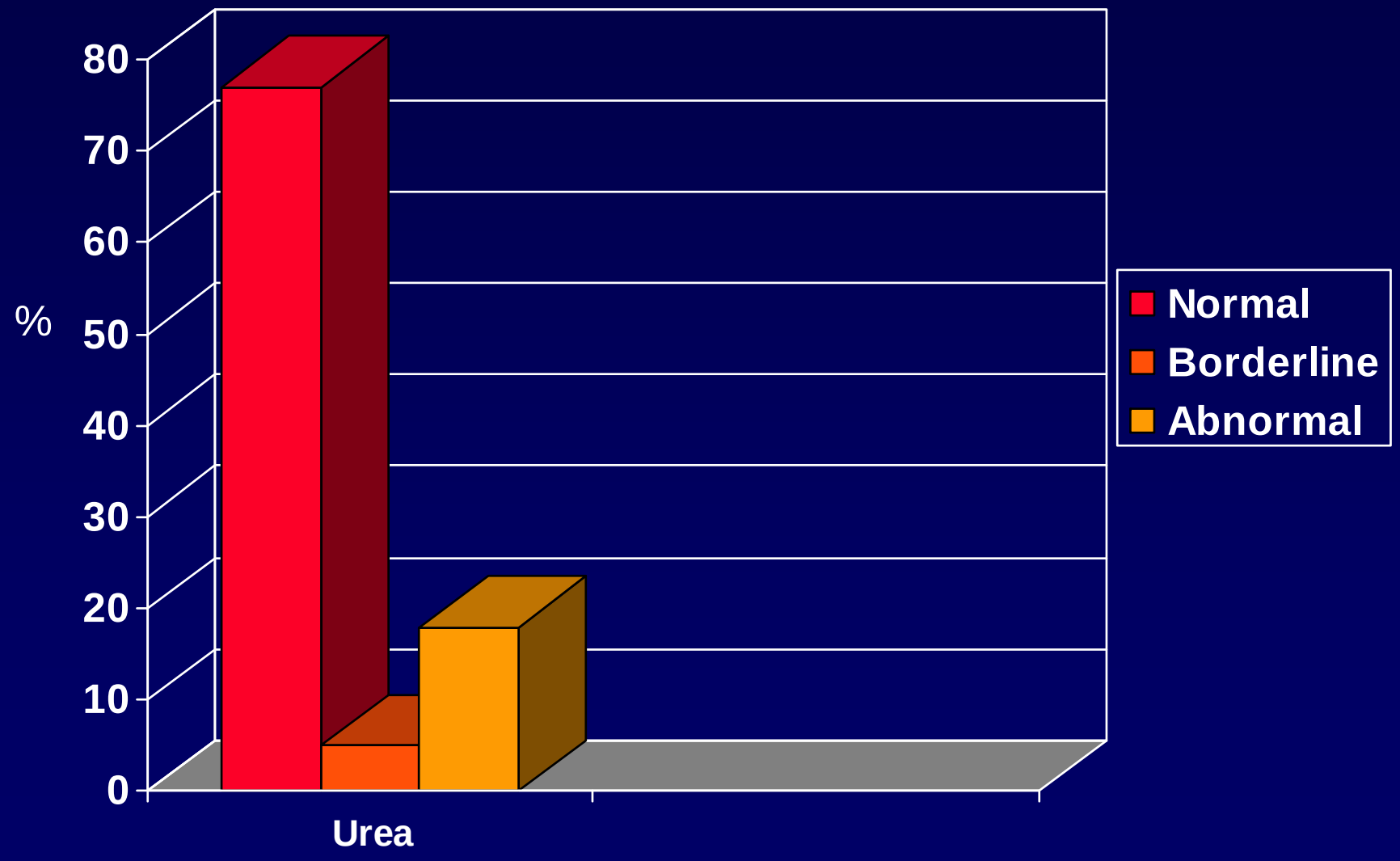


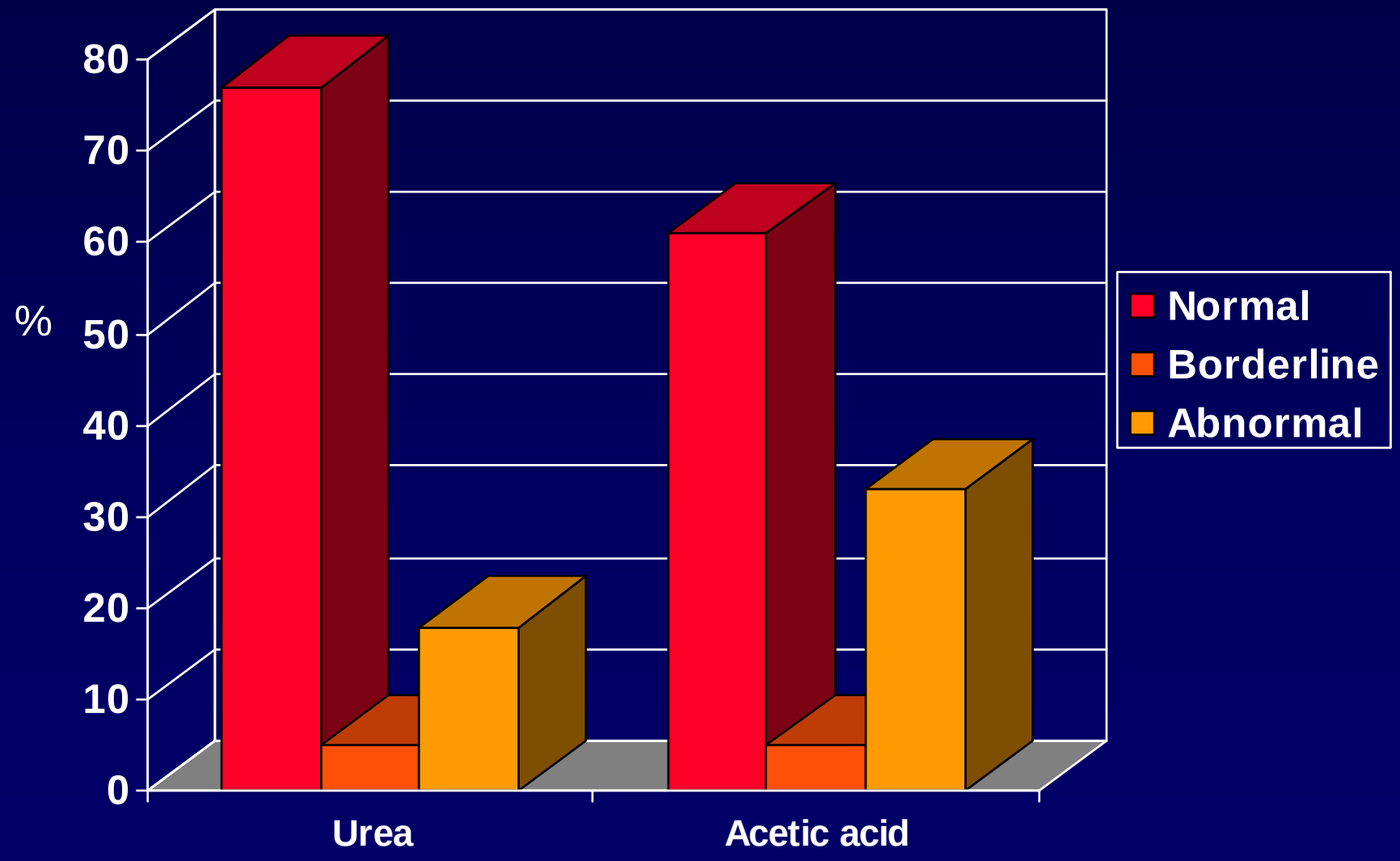
Factor XIII approx. 8u/dl

Interpretations by lysing agent



Factor XIII approx. 8u/dl

Interpretations by lysing agent



Relationship between FXIII solubility tests and FXIII assay levels

(in house ex vivo study)

Sample	FXIII level (u/dl)	Ca + Urea	Ca + A Acid	Thrombin + Urea	Thrombin + A Acid
XIII deficient (n=7)	<2 -4	+	+	+	+
XIII inhibitor (n=1)	8	-	+	+	+
XIII post treatment (n=2)	8-9	-	+/-	-	+

+ : positive screening test

Clot solubility:

Spiked FXIII deficient plasma

	Ca + Urea	Ca + A Acid	T + Urea	T + A Acid
0% FXIII	+	+	+	+
1% FXIII	+	+	+	+
5% FXIII	-	+	+	+
10% FXIII	-	-	-	+

+ : positive screening test

Conclusions

- Ca + Urea positive at 1u/dl
  - Negative at 5u/dl
- Ca + Acetic acid positive at 5u/dl
  - Negative at 10u/dl
- Thrombin + Urea positive at 5u/dl
  - Negative at 10u/dl
- Thrombin + Acetic Acid positive at 10u/dl

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- Why do only some thrombin-based screening tests detect mild factor XIII deficiency?
- Why do “identical” methods give different results?
- How accurate are factor XIII assays on FXIII deficient plasmas?

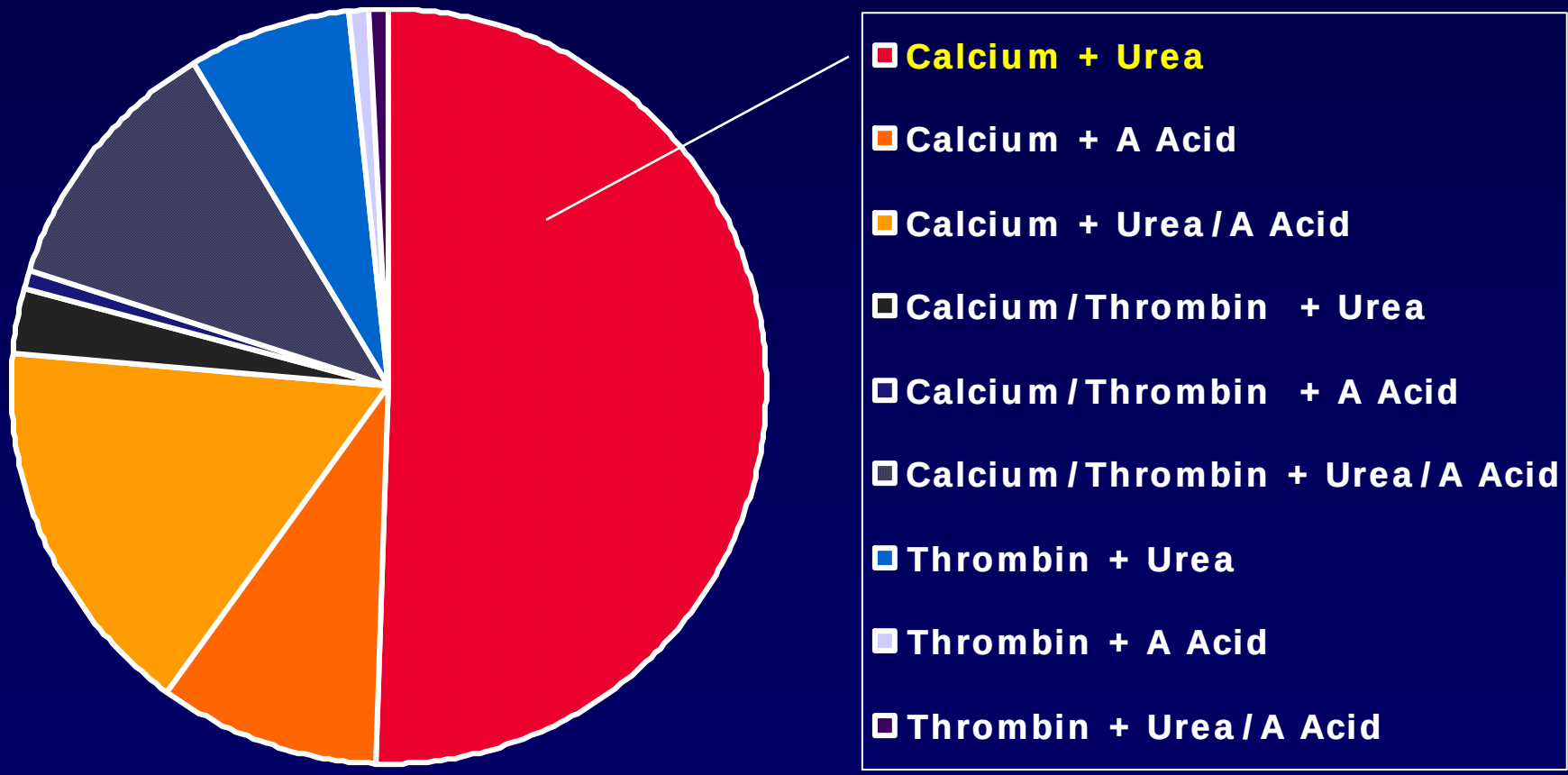
UK NEQAS:
Factor XIII screening

Variables:

- Ca conc. (20-25mM), Thrombin conc. (3.5- 50u)
- Incubation time (15 - 45 min)
- Incubation temp. (RT - 37oC)
- Urea conc. (5 - 10M)
- Volumes of plasma / reagents
- Normal ranges (0.5 - 24hrs)
- Source of method / normal range

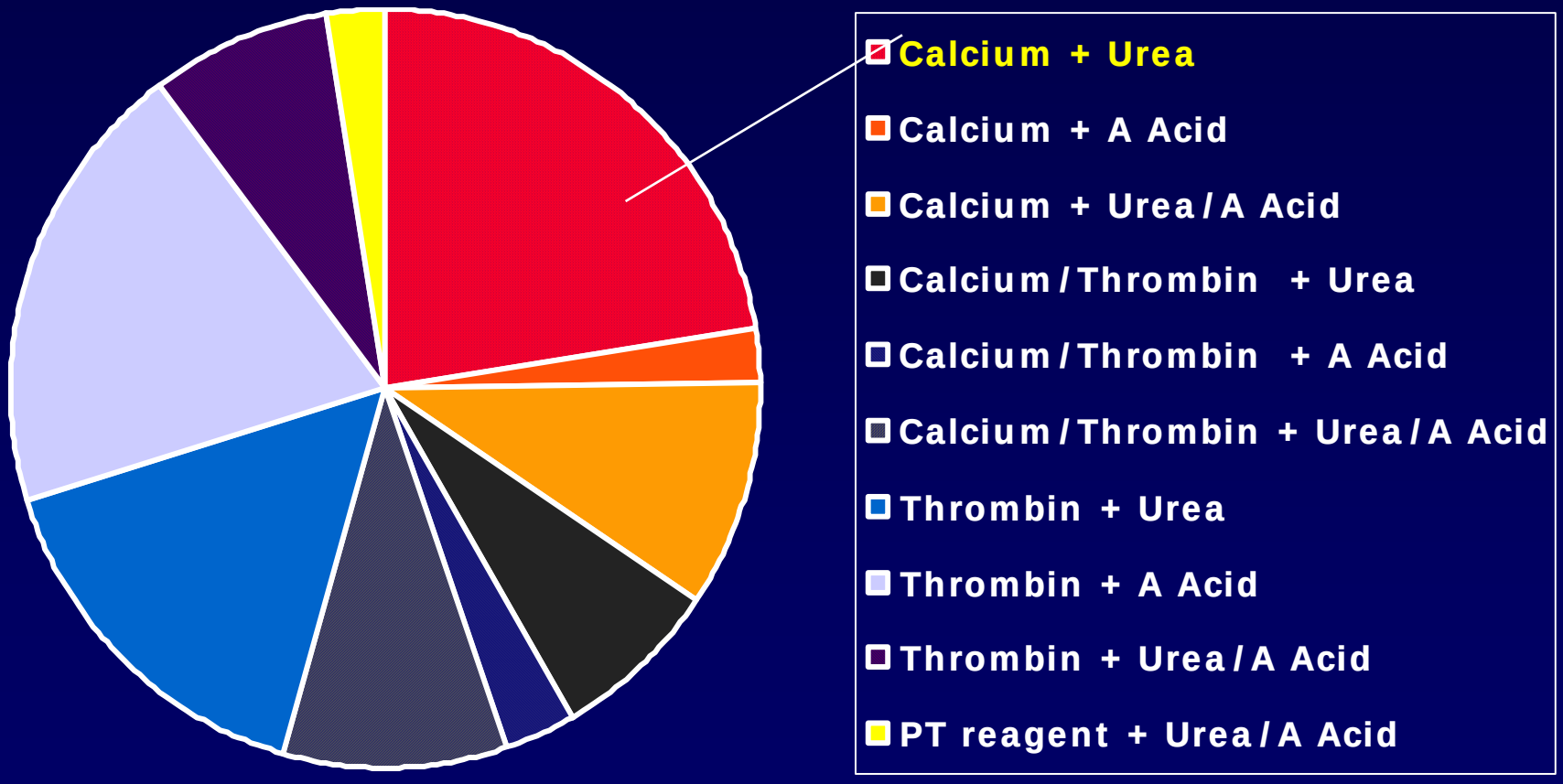
UK NEQAS: Methods in use

Factor XIII screening exercise, May 1997



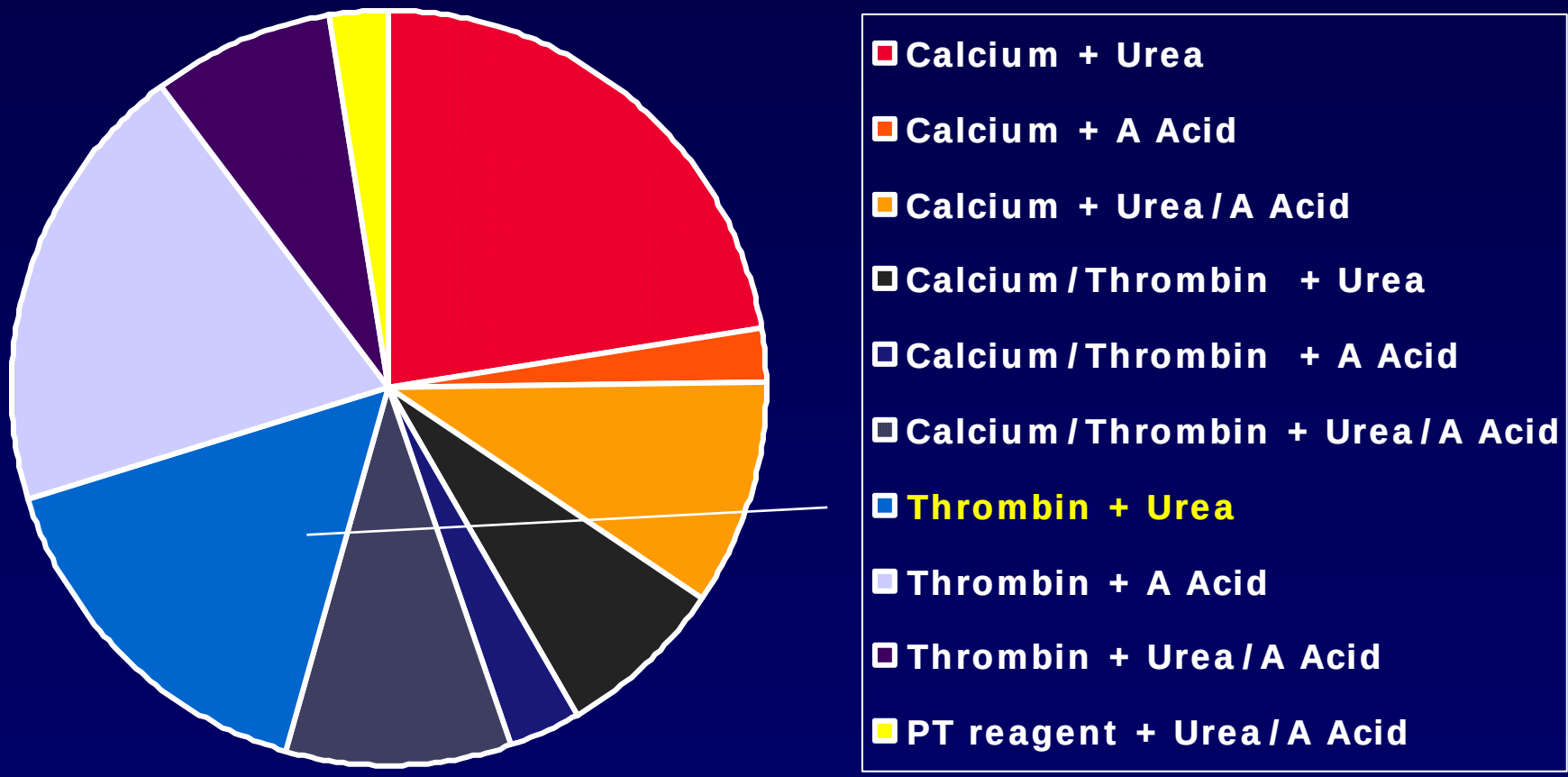
UK NEQAS: Methods in use

Factor XIII screening exercise, May 2002



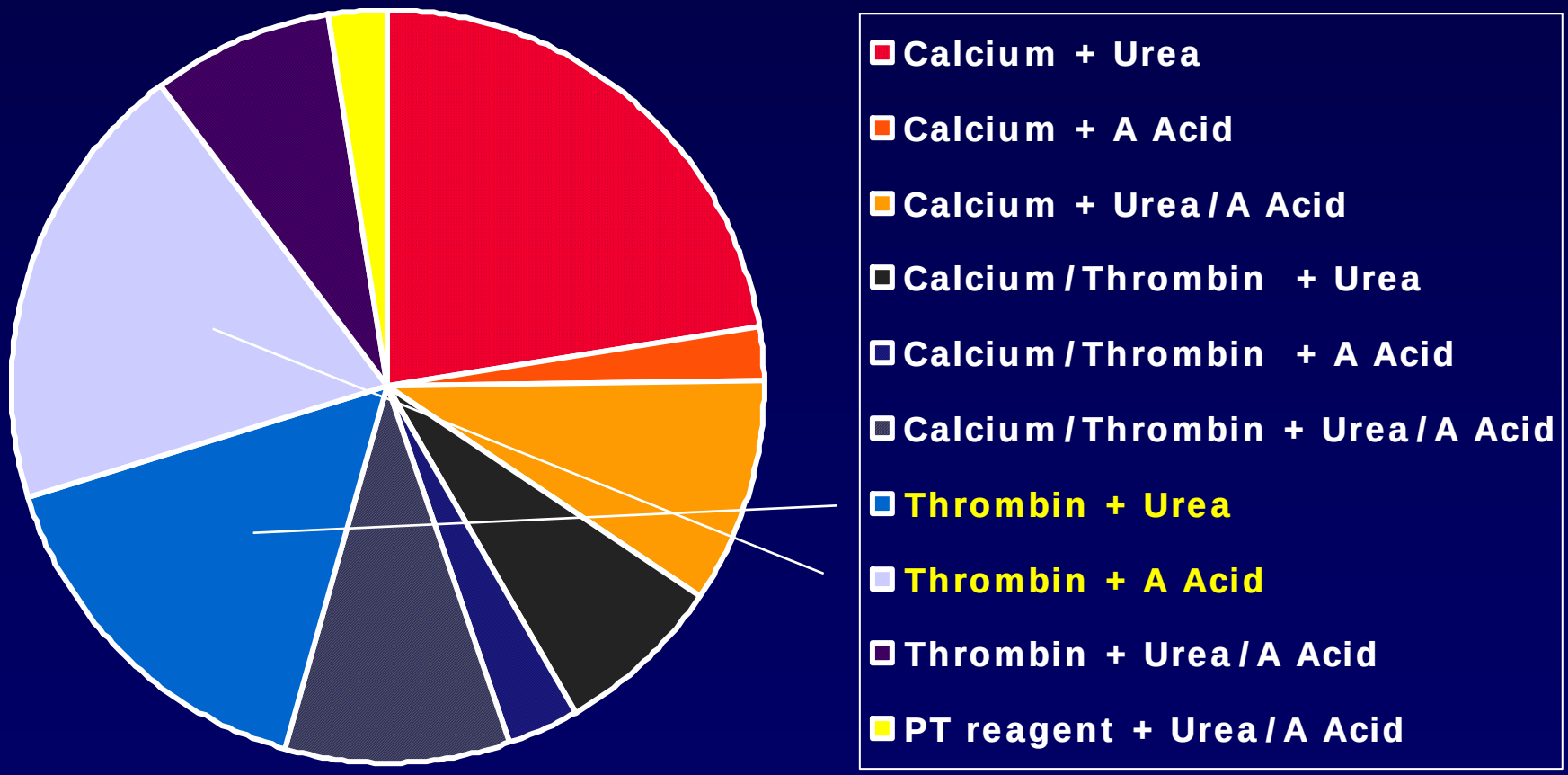
UK NEQAS: Methods in use

Factor XIII screening exercise, May 2002

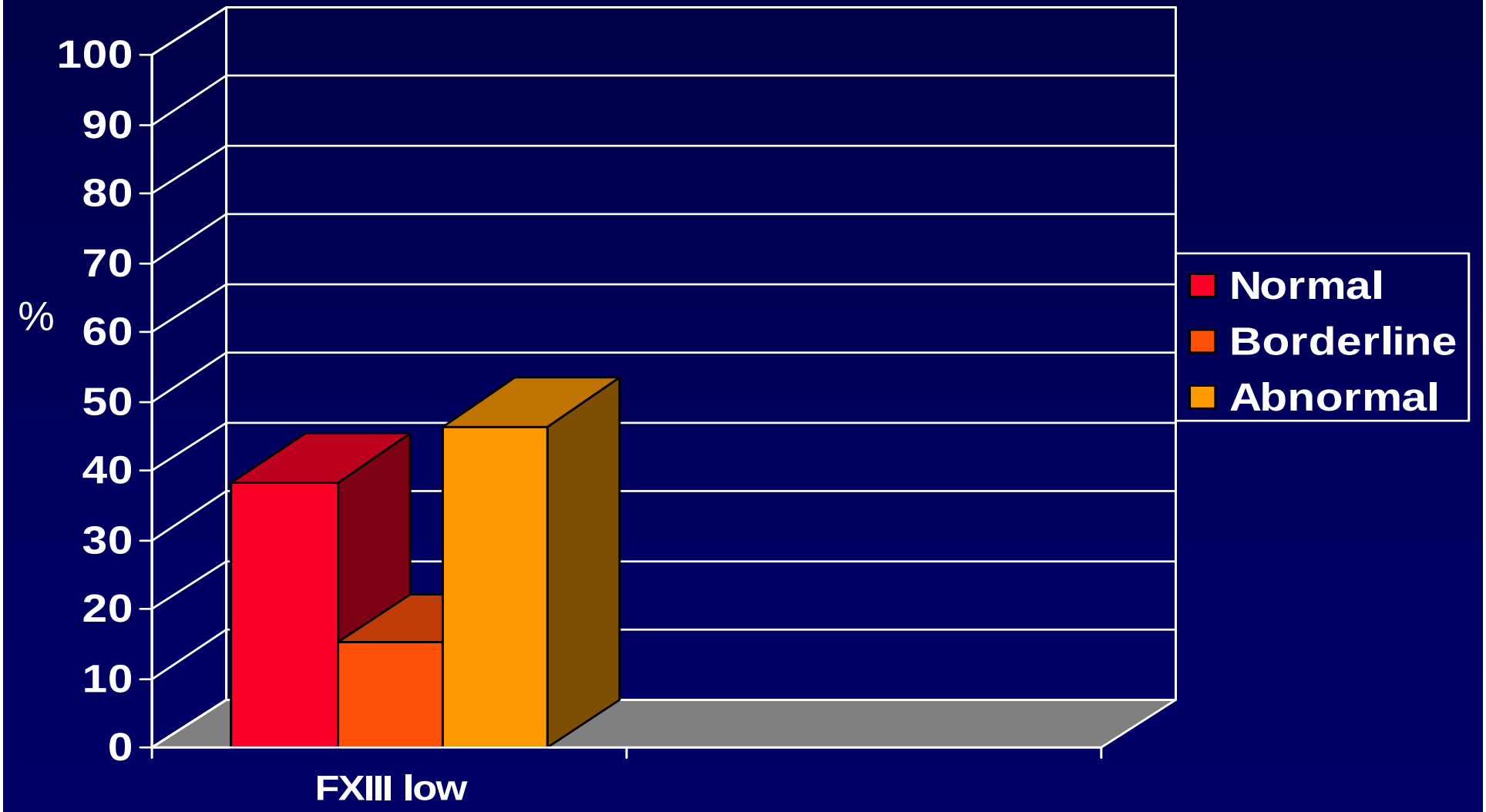


UK NEQAS: Methods in use

Factor XIII screening exercise, May 2002



Factor XIII screening exercise, May 2002
Interpretations



Factor XIII screening exercise, May 2002
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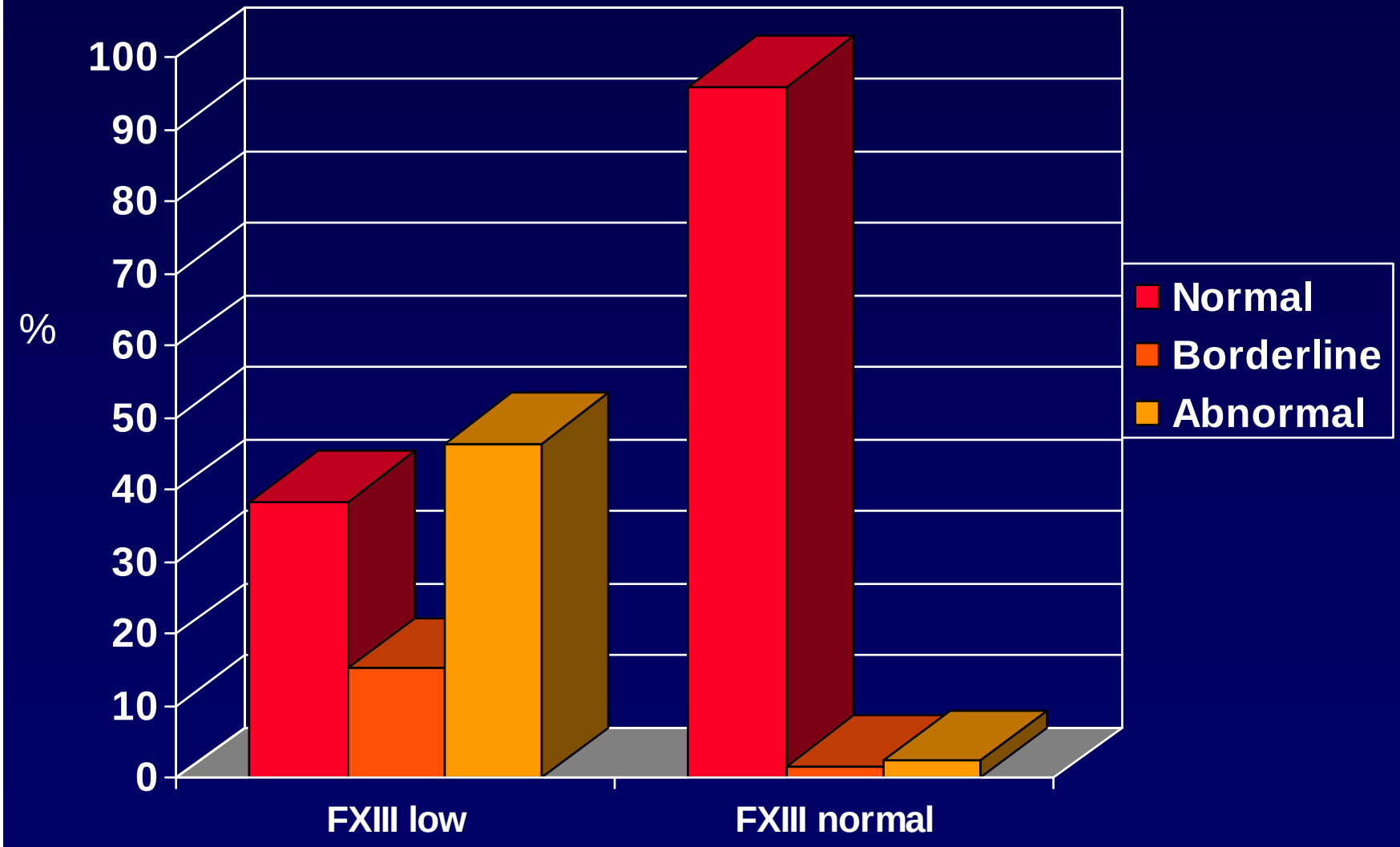
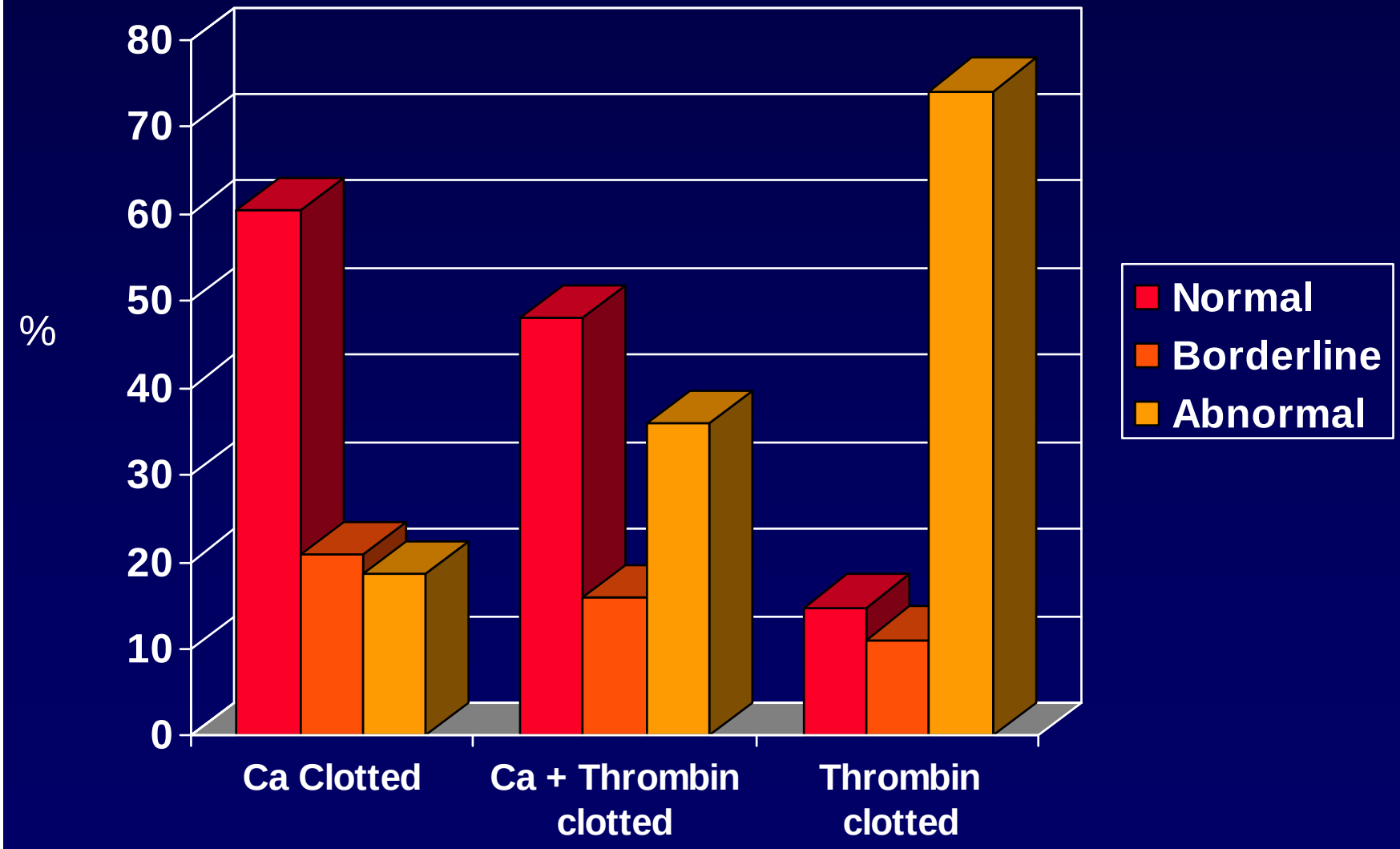


Interpretations by clotting agent: Low FXIII plasma



Thrombin-based screens: effect of reagent source

Source (n)	Range of conc. u/ml	Interpretations (%)		
		Normal	Borderline	Abnormal
A (1)	-	0	1	0
B (11)	6-15	3	1	7
C (17)	2-17	0	0	17
D (1)	5	0	0	1
E (10)	0.6-15	4	2	4
F (2)	7-15	0	0	2
G (2)	6	0	0	2
H (2)	5-7.5	0	1	1
I (3)	3.1-6.2	0	0	3

Thrombin – based screen tests
Why the difference?

- Some reagents contain calcium

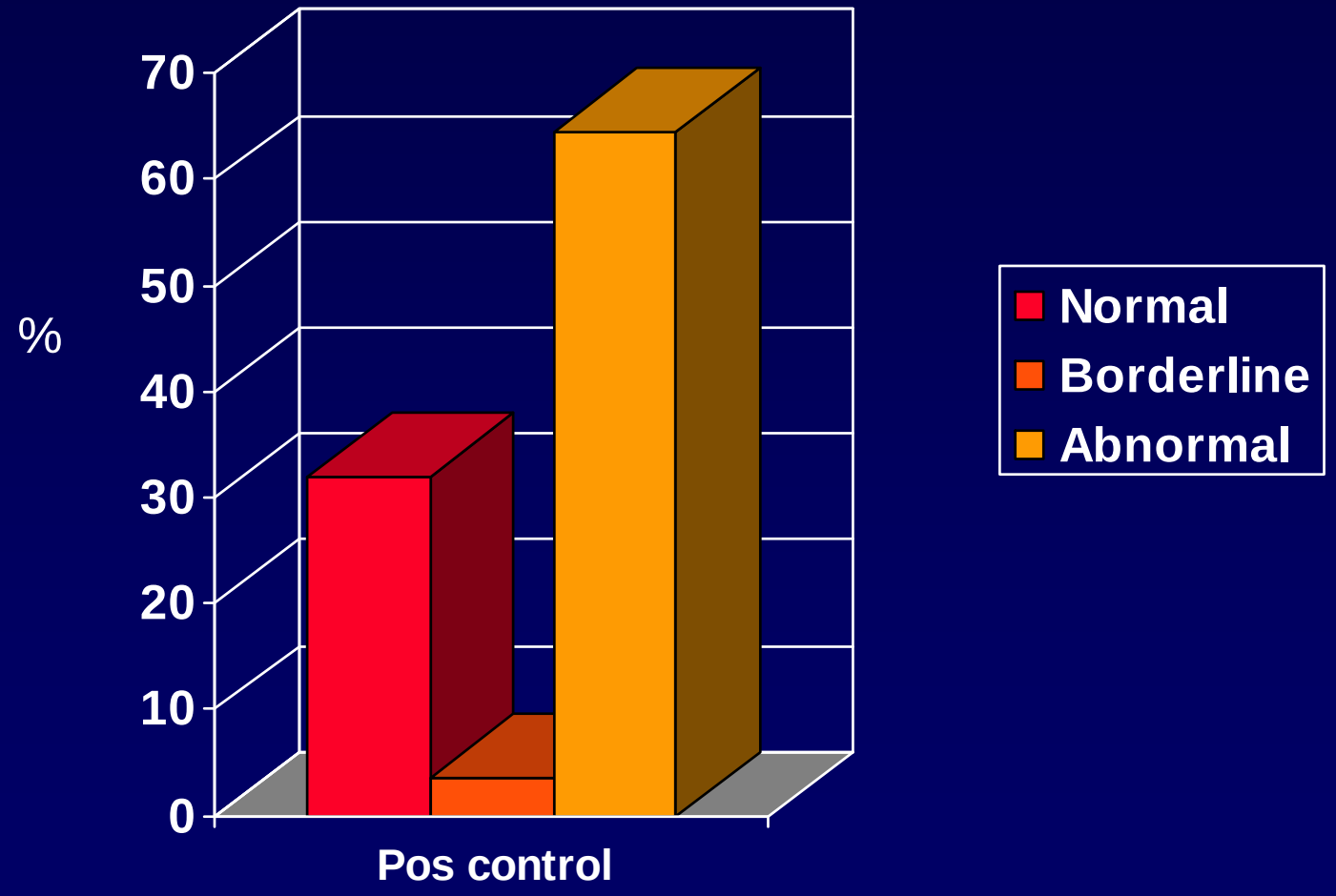
UK NEQAS FXIII Screening Exercise:

Positive Control Plasma

- Blood collected into EDTA anticoagulant
- Clot with thrombin (equal volume 20u/ml)
- Leave at 37^{o} C, 30mins
- Add 5M urea or 2% acetic acid
- Incubate at RTo C
- Check at 30min intervals for clot lysis

UK NEQAS FXIII Screening Exercise:

Positive Control Plasma: Interpretations

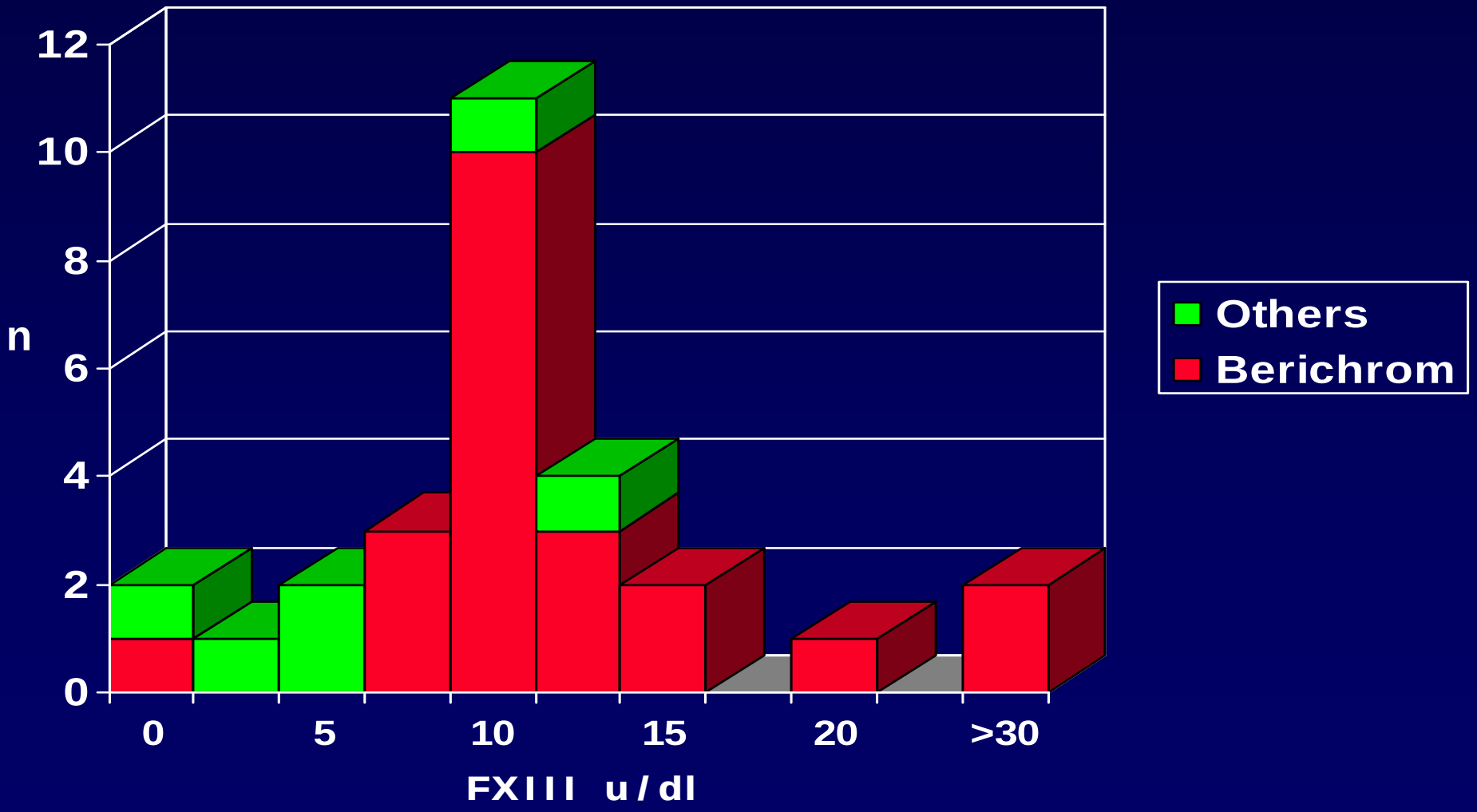


Thrombin-based screens: effect of reagent source EDTA “positive control”

Source	Interpretations (%)		
	Normal	Borderline	Abnormal
A	2	0	0
B	0	0	1
C	2	1	10
D	1	1	24
E	0	0	1
F	7	0	5
G	2	0	1
H	0	0	1
I	0	0	2
J	0	0	2
K	0	0	3

FXIII assays: Low FXIII plasma

Median 10.1u/dl, range 0-55u/dl



International Registry on Factor XIII Deficiency (European)

Ivaskevicius et al 2007

“There was no strong correlation between the severity of bleeding and FXIII levels. This might be partly due to inaccuracy of FXIII measurement levels”

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- **How much factor XIII is required for normal haemostasis?**

- ***1974 Jackobsen & Godal***
  - 2-3% minimum required for haemostasis
  - clot soluble in urea until 3-5% NP
  - in absence of Ca++, soluble until 25-30% NP
- ***1977 Losowsky & Miloszewski***
  - as little as 1% or as much as 5% required for haemostasis
- ***1996 Seitz et al***
  - 7/14 patients with FXIII levels between 5-40% suffered severe bleeding symptoms

Factor XIII screening: Conclusions

- Increased sensitivity of thrombin-based screening tests is dependant on the source of thrombin
- This is also important if using EDTA plasma as a positive control
- Lack of accuracy & precision is still evident for FXIII assays
- The clinical relevance of “mild” FXIII deficiency is unresolved

A collaborative study to establish the 1st International Standard for factor XIII plasma

"The preparation was proposed to and accepted by the Expert Committee on Biological Standardisation of WHO to be the 1st IS for FXIII plasma with an active potency of 0.91 IU per ampoule and an antigen potency of 0.93 IU per ampoule"

Raut et al 2007