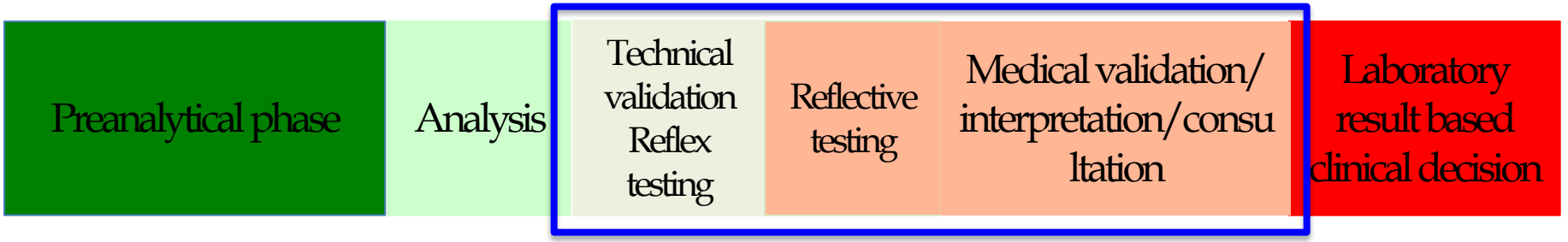
How do laboratories interpret unexpected APTT prolongation?

Post-analytical external quality assurance survey
in laboratories of 35 countries

Ajzner E, Rogic D, Meijer P, Kristofersson AH, Somen E, Watine J, Sandberg S

Working Group on Postanalytical External Quality Assurance
(**http://efcclm.eu/science/wg-postanalytical-external-quality-assurance**)
in the Science Committee on European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) and European Organisation for External Quality Assurance Providers in Laboratory Medicine (EQALM)

Post-analytical actions in interpretation of APTT prolongations



I. exclude **preanalytical errors**
II. consider the influence of **therapies**
III. perform **mixing study** as a decision test in first level diagnosis: presence of inhibitor or factor deficiency
IV. apply **special coagulation tests in further investigations** –based on first level diagnosis and clinical history- for diagnosis making

GOALS OF THE STUDY

To get insight into the existing post-analytical procedures (max. until first level diagnosis is made) that are induced by unexpected APTT prolongation.

Based on the results we hope to better understand:

- the range of existing practice variations
- interpretative thinking and skill of laboratories (step-by-step investigation protocols, misinterpretations)
- the available guidelines and their utilization in everyday practice

Case history

A 7-year old girl has suffered from gastroenteritis which lasted for three days with fever, vomiting and mild diarrhea. Two weeks later, her citrated blood sample was delivered to your laboratory as a part of a general checkup before elective tonsillectomy.

The results were:

PT: 11,2 sec (reference interval: 9-12 sec)
INR: 0,98 (reference interval: 0,8-1,2)
APTT: 65,0 sec (reference interval: 28,0-35,0 sec)

Structure of questions subsequent to case history

EXCLUSION OF **PREANALYTICAL ERRORS** **(I.)**

CONSIDERATION OF **THERAPIES** **(II.)** resulting in APTT prolongation

MIXING STUDIES **(III.)**

Mixers

Non-mixers

TECHNICAL DETAILS OF MIXING STUDY:

- What is the source of normal plasma in the mixture?
- Buffered or non buffered?
- Ratio of patient and normal plasma in the mixture?
- Incubate or not?
- How long do you incubate?

INTERPRETATION OF MIXING STUDY

- in theory
- in practice: 3 laboratory scenarios

FURTHER INVESTIGATIONS **(IV.)**

REPORTING **(V.)**

The survey design

- The short case report with single and multiple choice questions were adapted on web using SurveyMonkey**https://www.surveymonkey.com/s/APTT-interpretation_and_testing**

- Invitations containing access link to the survey were sent to laboratories performing APTT with help EQALM and ECAT.

- Persons responsible for coagulation in each laboratory were asked to answer.

- Pilot version: April 2012
- Launch: June 2012 End: 31st October 2012
- Presentation until the end of September 2012

PARTICIPANTS

- Laboratory invitations in 35 countries
- 16 countries provided 95% of all responses until the end of September

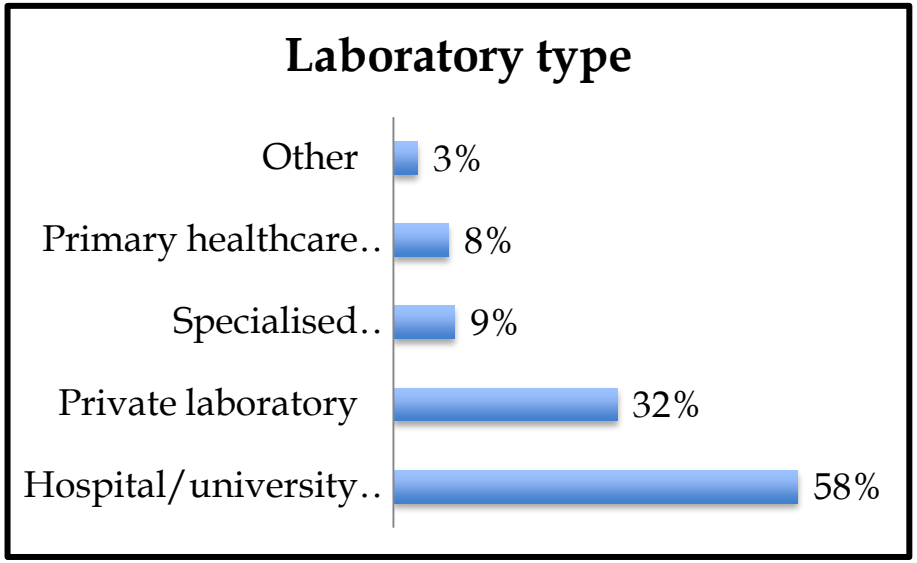
	Responses	invitations	response rate
France	234	1000	23%
Italy	107	280	38%
The Netherlands	70	129	54%
Portugal	68	88	77%
Germany	49	760	6%
Austria	46	270	17%
Switzerland	46	211	22%
Hungary	45	160	28%
Ireland	37	54	69%
Norway	35	73	48%
Croatia	34	199	17%
The Czech Republic	30	458	7%
Denmark	18	NA	NA
The United States	15	65	23%
Russia	11	515	2%
Sweden	9	19	47%
Total	**854**	**4281**	**20%**

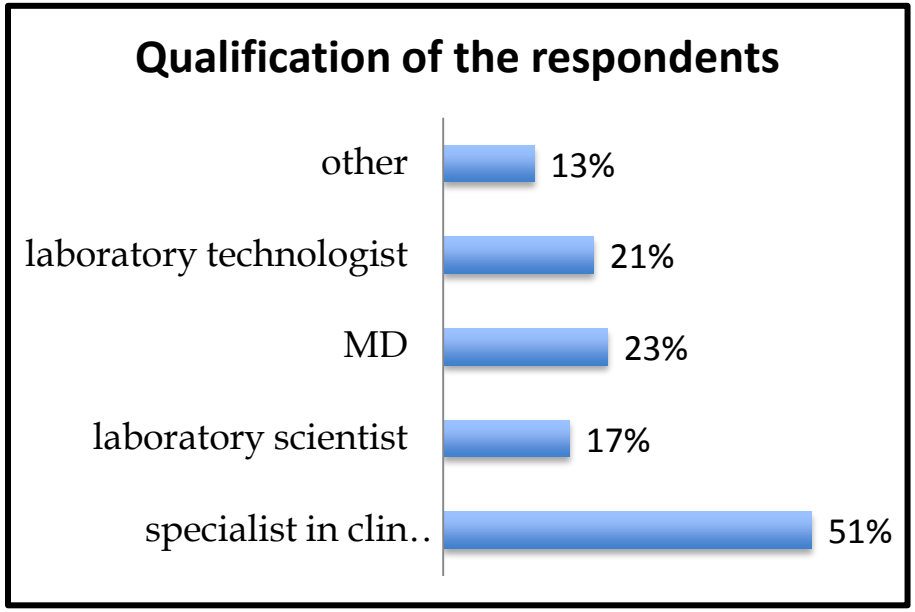
Laboratory and practice particulars

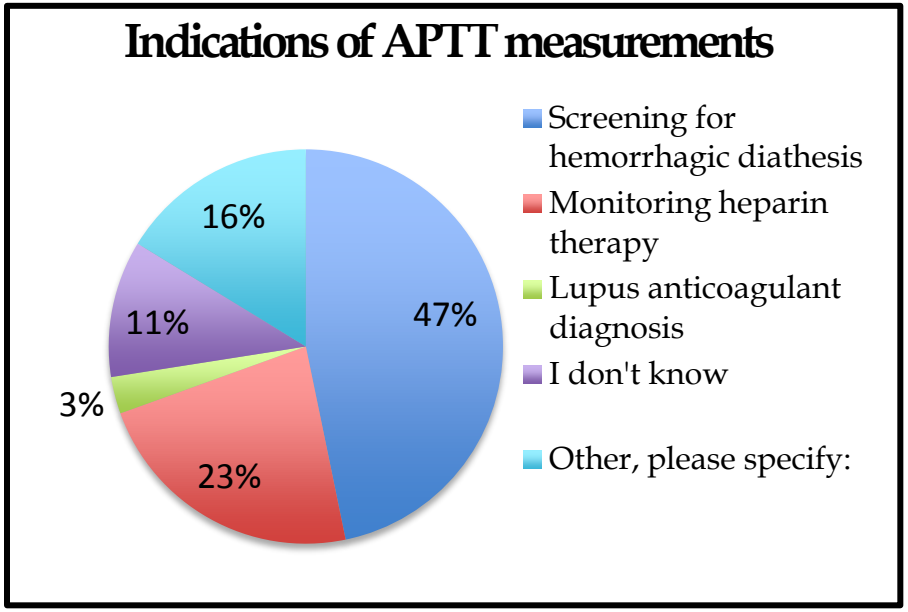
Size: Median no of daily APTT-s: 40 (min-max: 0-1000)

Total no of answerers: 902
(37% male, 63% female)

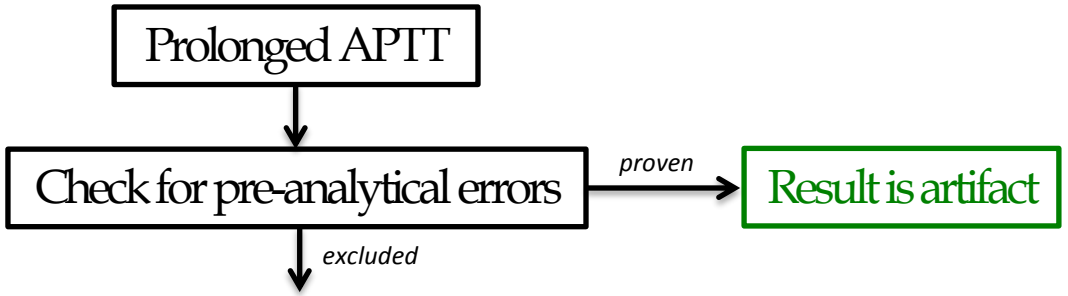
Median age:
48 years (min-max: 20-70)



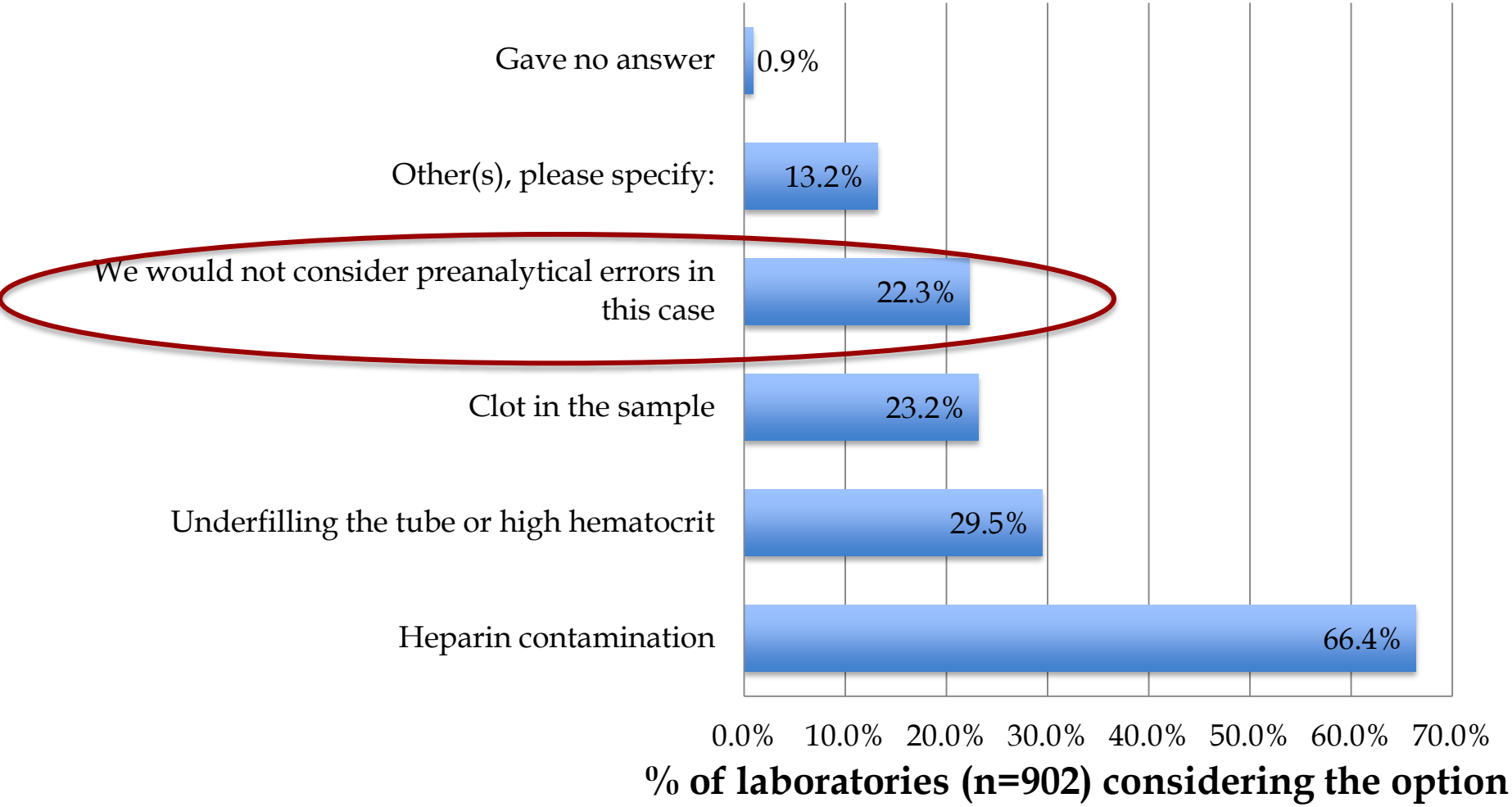




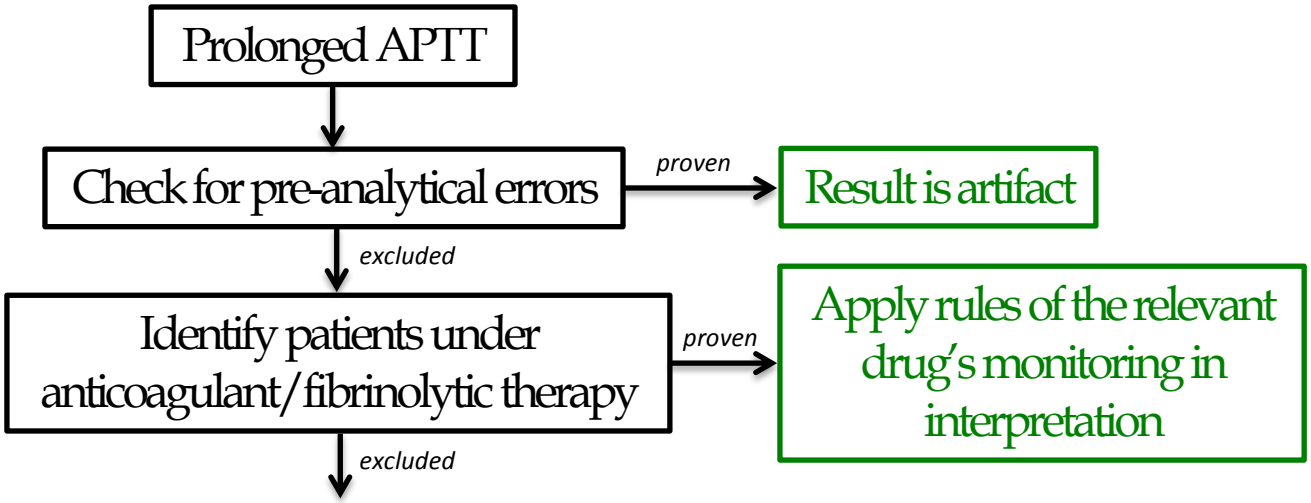
Post-analytical actions to achieve first level interpretation of APTT prolongations



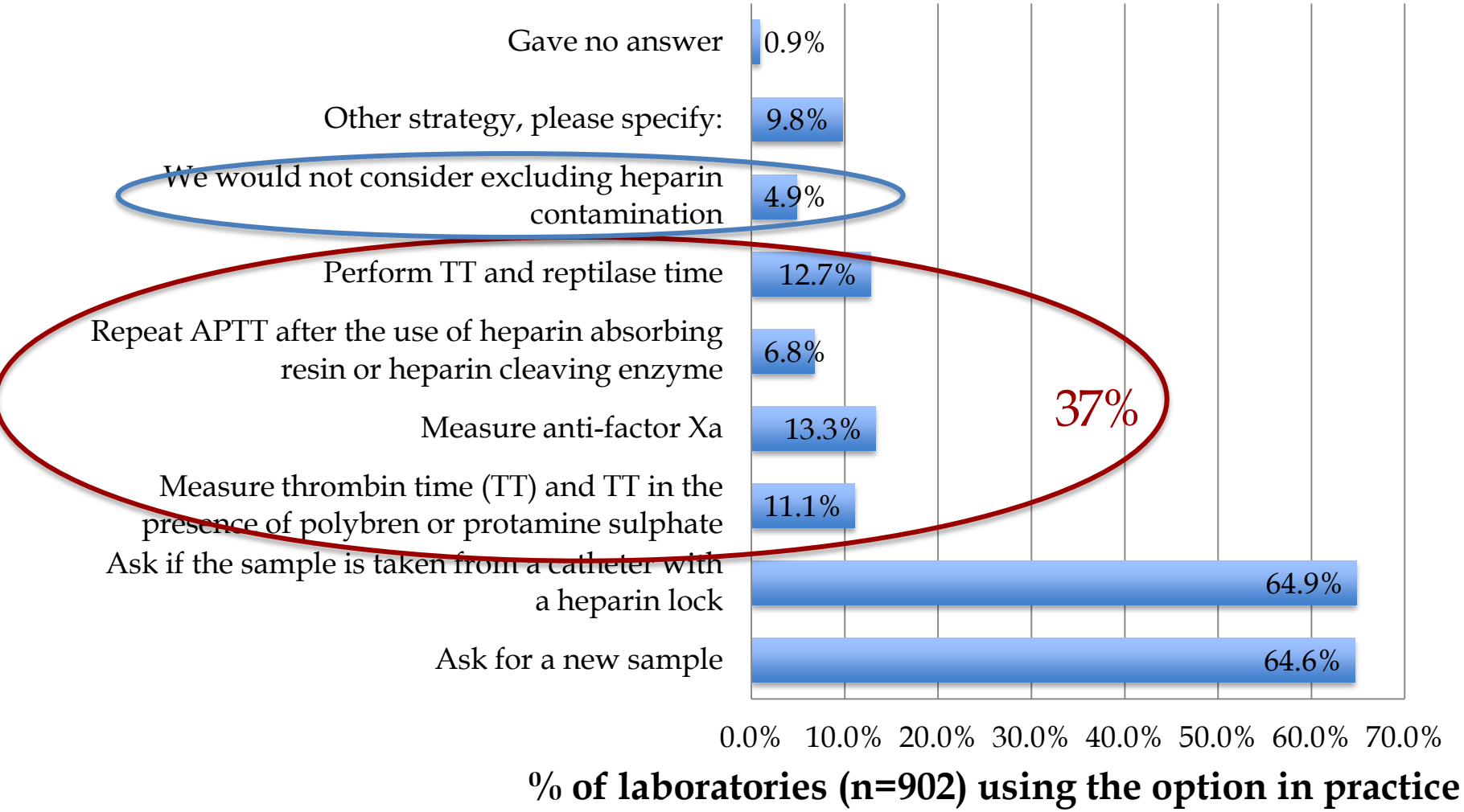
Which preanalytical errors would you consider in this case?



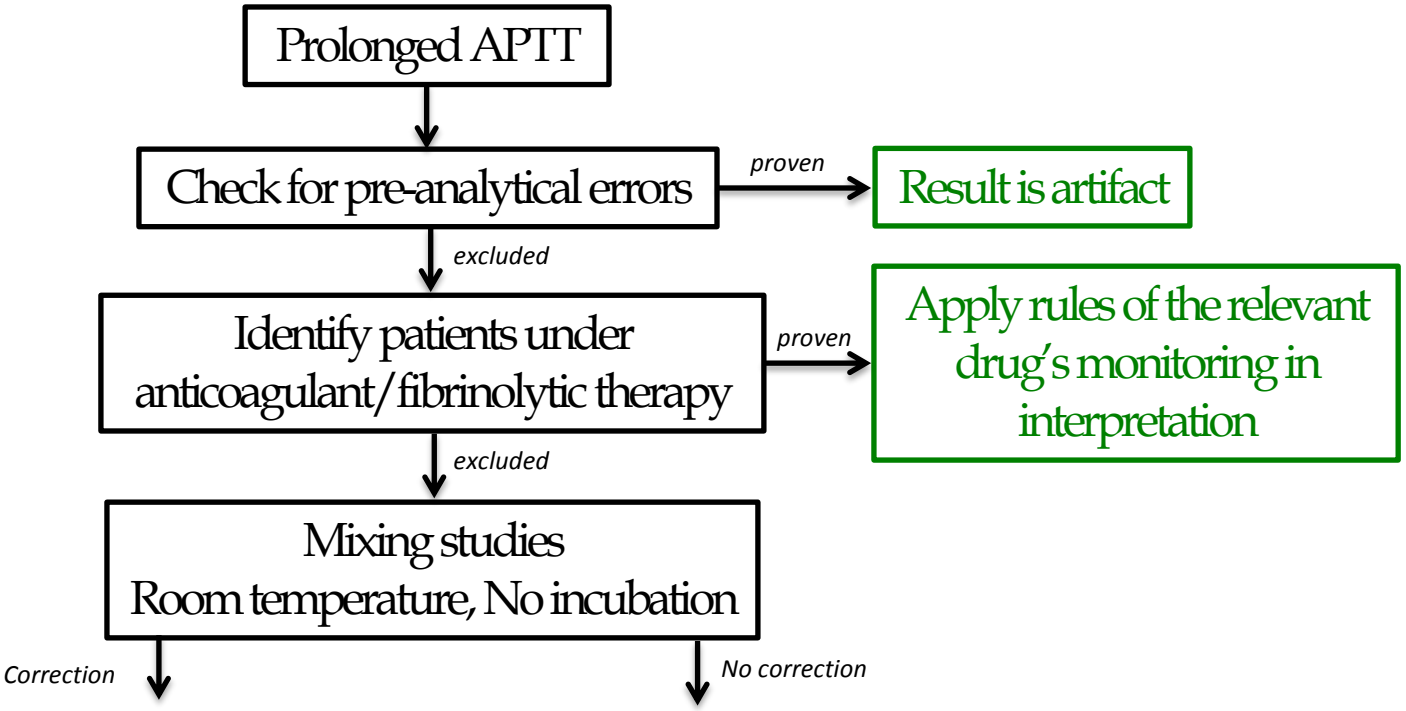
Post-analytical actions to achieve first level interpretation of APTT prolongations



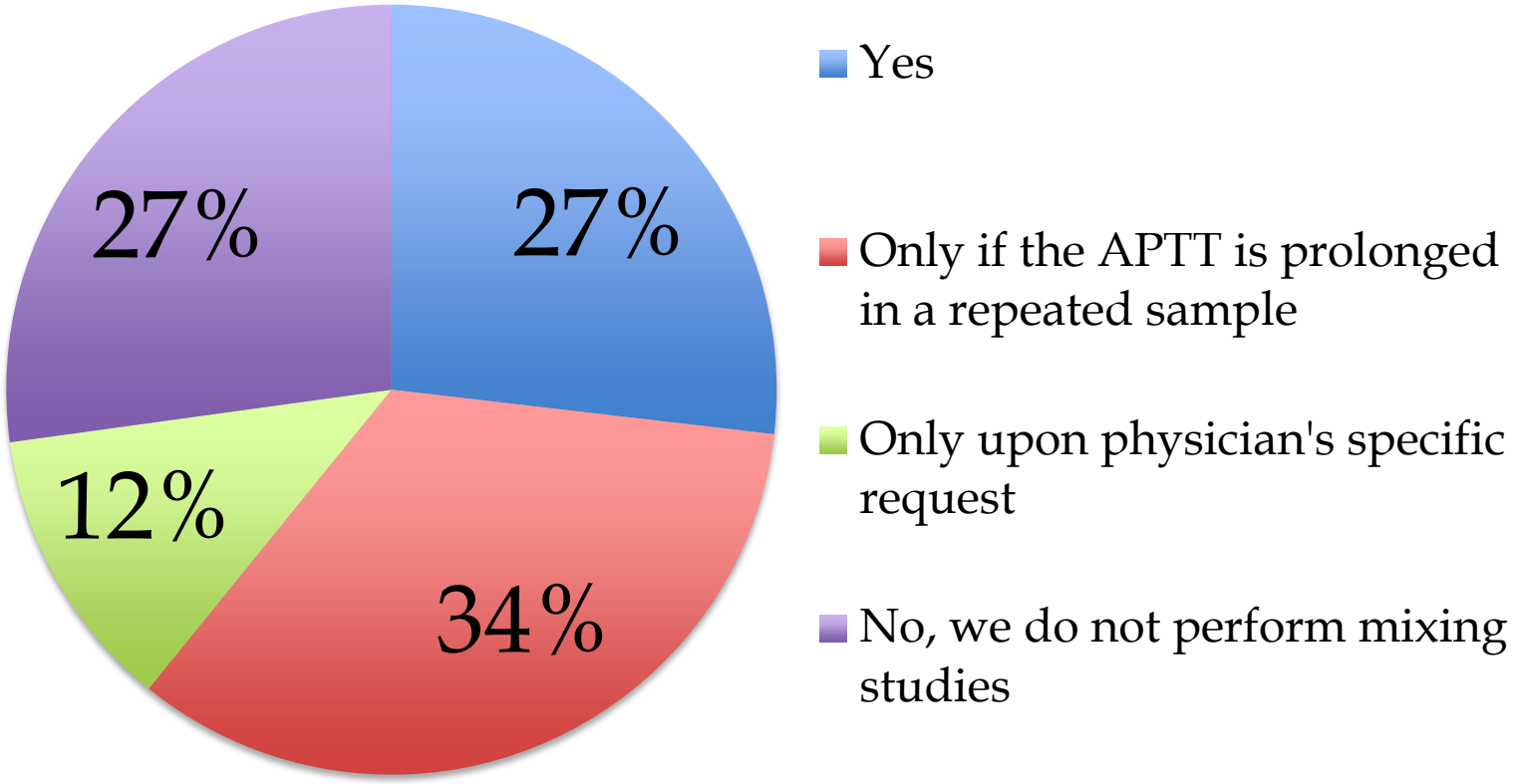
Methods used for exclusion heparin presence in the sample: What do you usually do in your laboratory to exclude heparin contamination?



Post-analytical actions to achieve first level interpretation of APTT prolongations

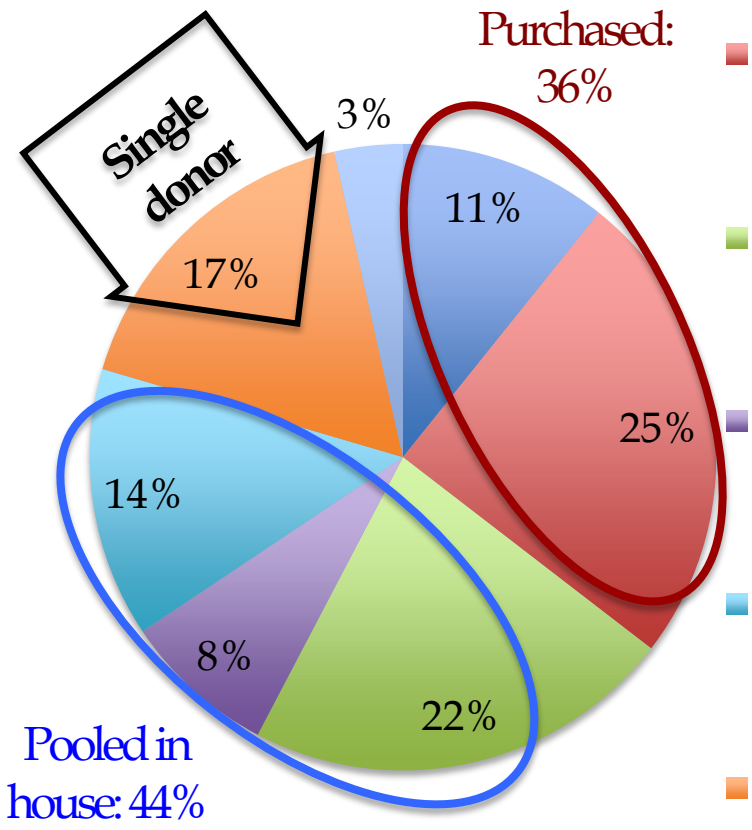


Would you perform APTT mixing studies in this case?



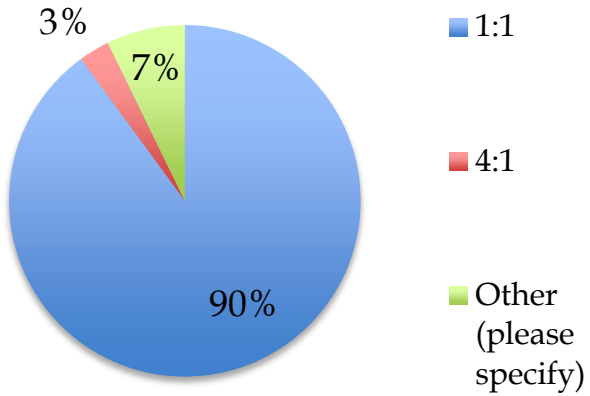
Technical details of APTT mixing studies

SOURCE OF NORMAL PLASMA

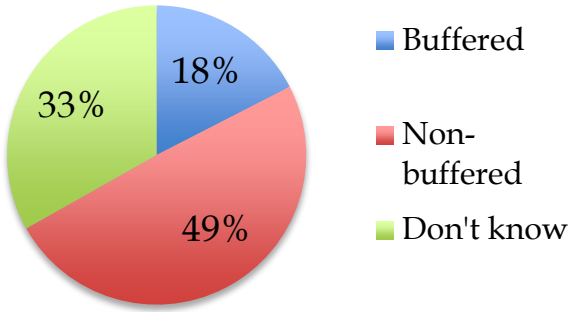


- Purchased frozen normal control plasma
- Purchased lyophilised normal control plasma
- Pooled fresh citrated plasma collected from healthy donors
- Frozen (-20 C) pooled citrated plasma collected from apparently healthy donors
- Frozen (-80 C) pooled citrated plasma collected from apparently healthy donors
- Fresh citrated plasma from a single healthy donor

RATIO IN THE MIXTURE

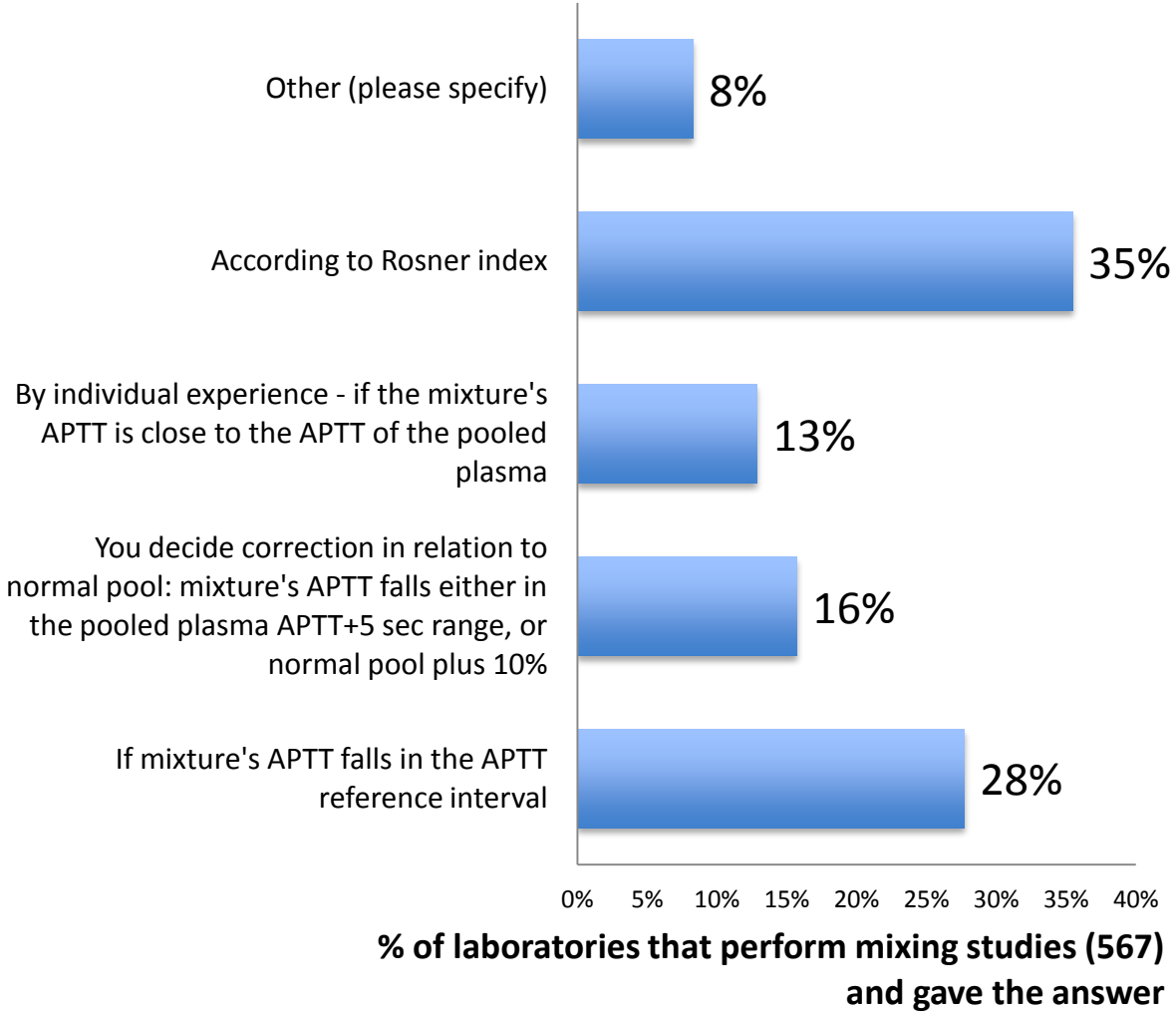


BUFFERED CONDITONS



Interpretation of mixing study: theory

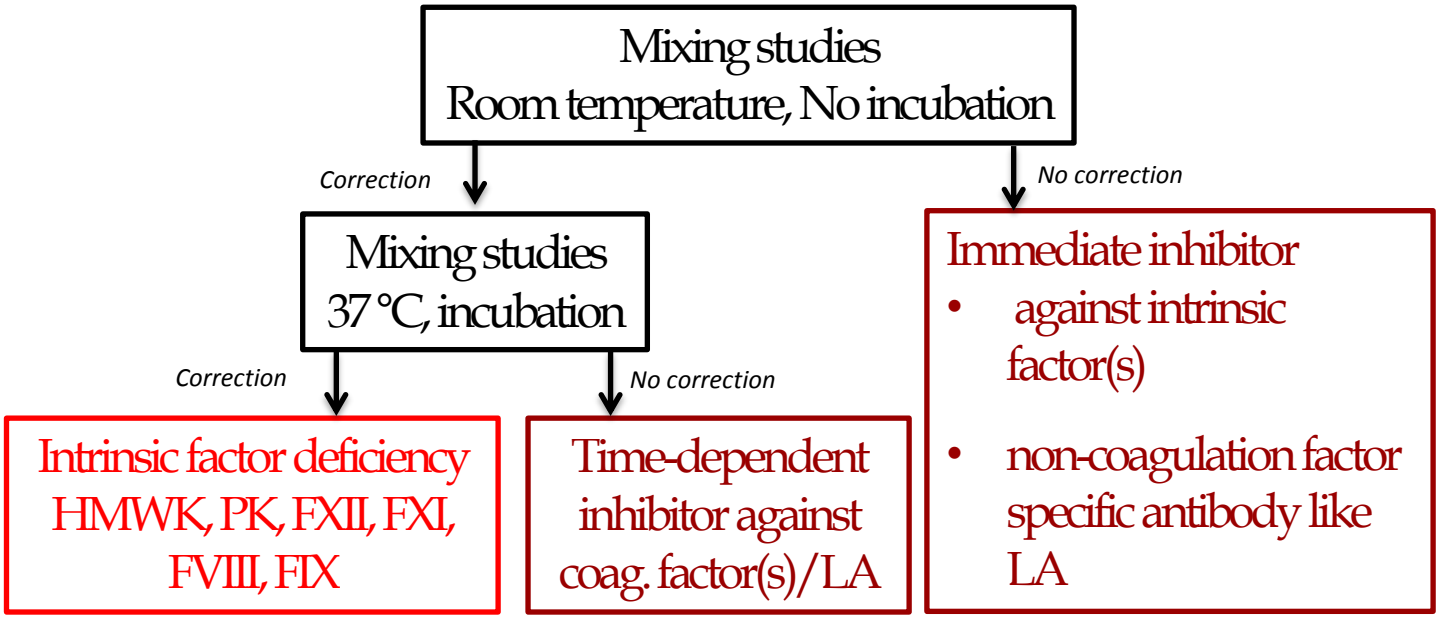
When do you classify the results of mixing studies to be indicating factor deficiency (no inhibitor)?



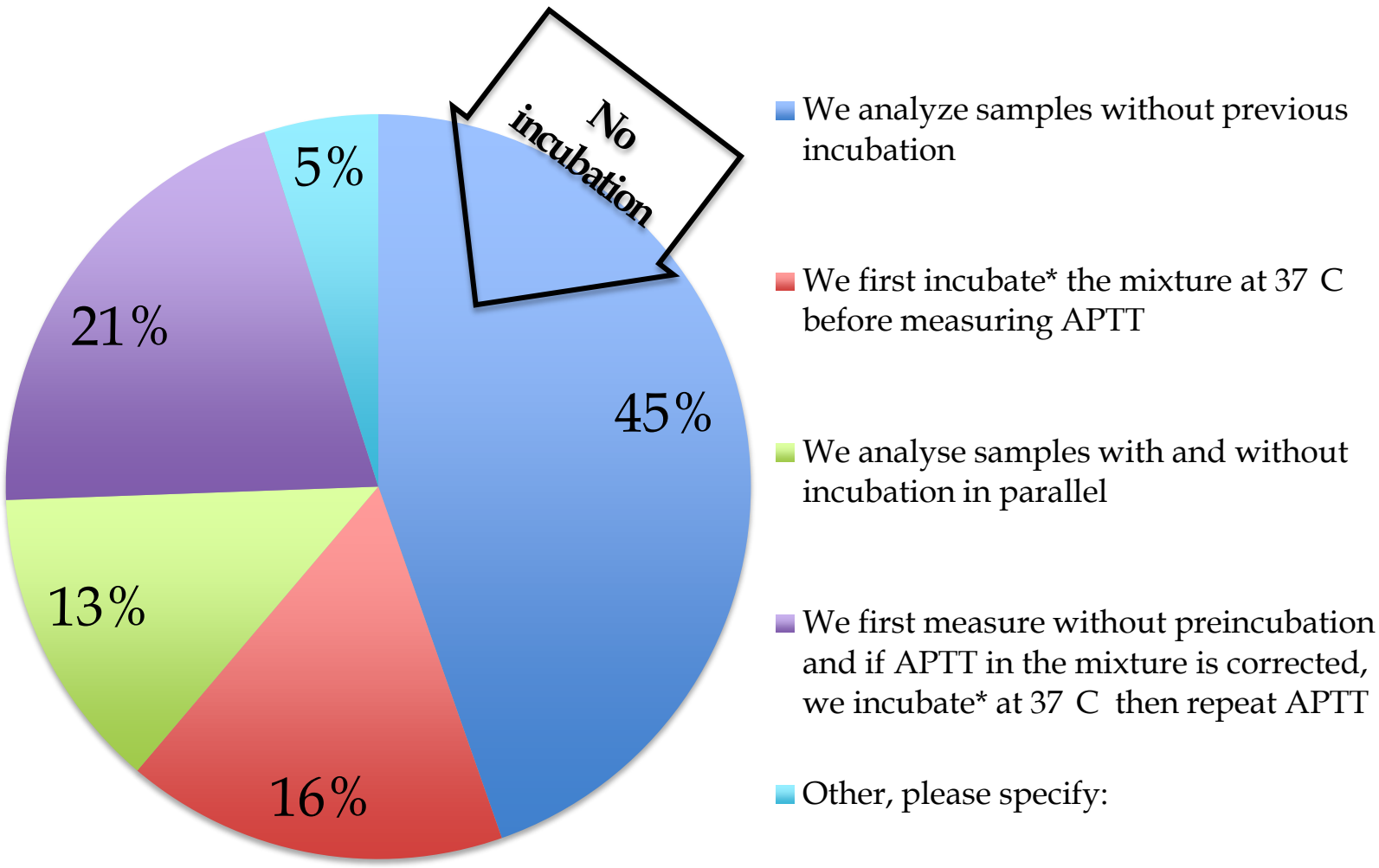
Incubation at 37°C (1 or 2 hrs)

Correction of the immediate mix *does not rule out* the presence of an inhibitor, since FVIII inhibitors and some LAs display time dependency.

CLSI guideline
H47-A2, 28;20, 2008



Incubation of samples in APTT mixing studies



Interpretative skill

- Different **mixing study patterns** (scenario A, B, C)

- **Potential interpretations (multiple choice)**

  Coagulation factor deficiency (no inhibitor)

  Presence of coagulation factor specific inhibitor type I or type II

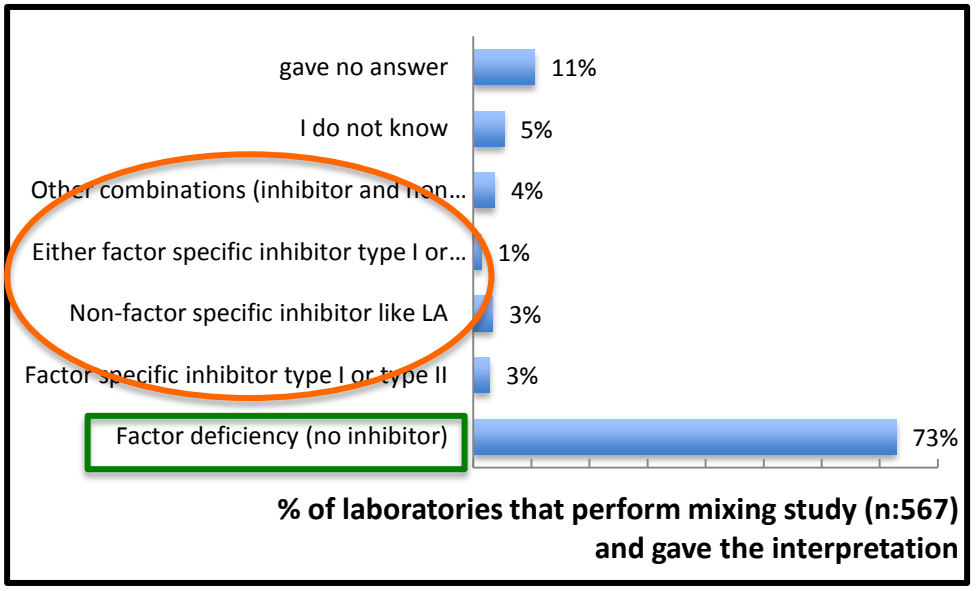
  Presence of non-coagulation factor specific inhibitor like lupus anticoagulant

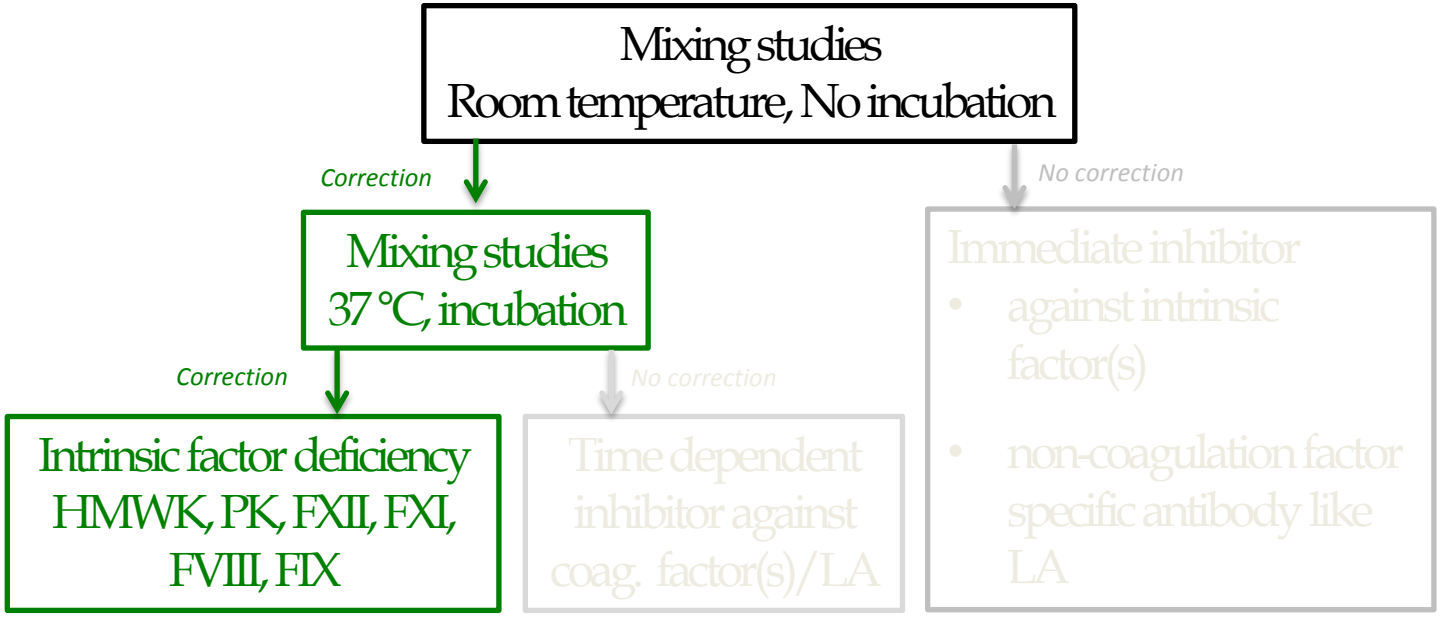
  I do not know

- Investigated
  - if laboratories can successfully interpret **different result combinations** of immediate and time-dependent mixing studies
  - if they are aware of the fact **that mixing studies can differentiate only between lack and presence of inhibitors but not between inhibitor types**

Interpretation of mixing study
Scenario B: intrinsic factor deficiency, no inhibitor

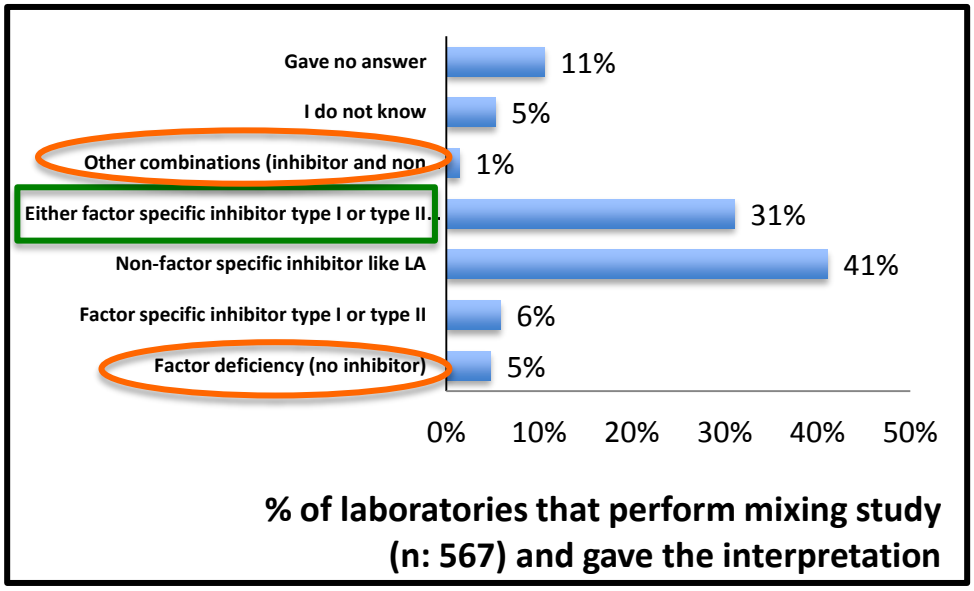
APTT 1:1 mixture of patient and pooled plasma	33 sec
APTT measured on pooled plasma	32 sec
APTT 1:1 mixture of patient and pooled plasma at 37°C	38 sec
APTT measured on pooled plasma at 37°C	35 sec





Interpretation of mixing study
Scenario A: immediate inhibitor

APTT 1:1 mixture of patient and pooled plasma	56 sec
APTT measured on pooled plasma	32 sec
APTT 1:1 mixture of patient and pooled plasma at 37°C	59 sec
APTT measured on pooled plasma at 37°C	35 sec



% of laboratories that perform mixing study (n: 567) and gave the interpretation

Mixing studies
Room temperature, No incubation

Correction

Mixing studies
37 °C, incubation

Correction

Intrinsic factor deficiency
HMWK, PK, FXII, FXI, FVIII, FIX

No correction

Time dependent inhibitor against coag. factor(s)/LA

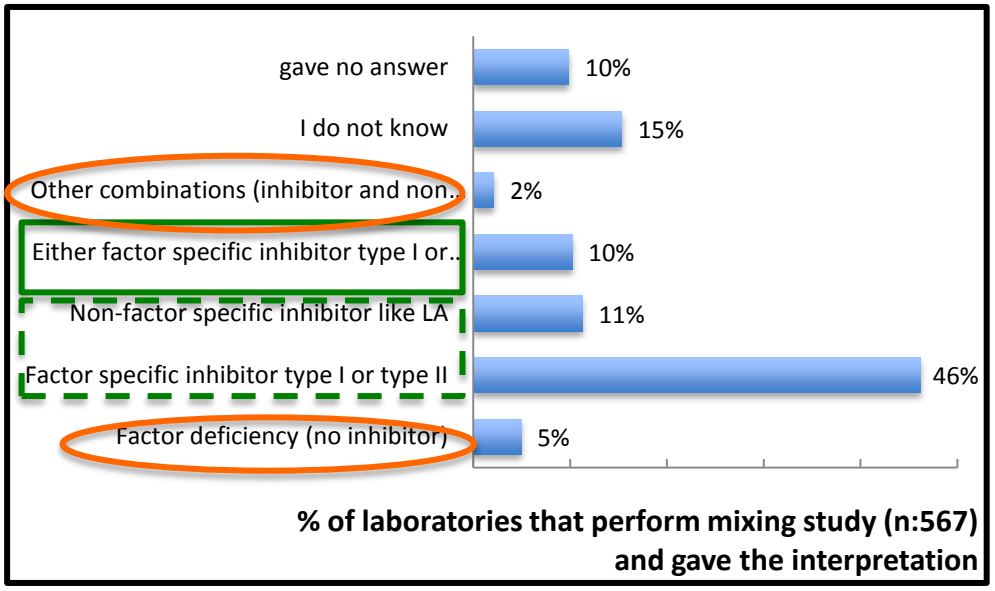
No correction

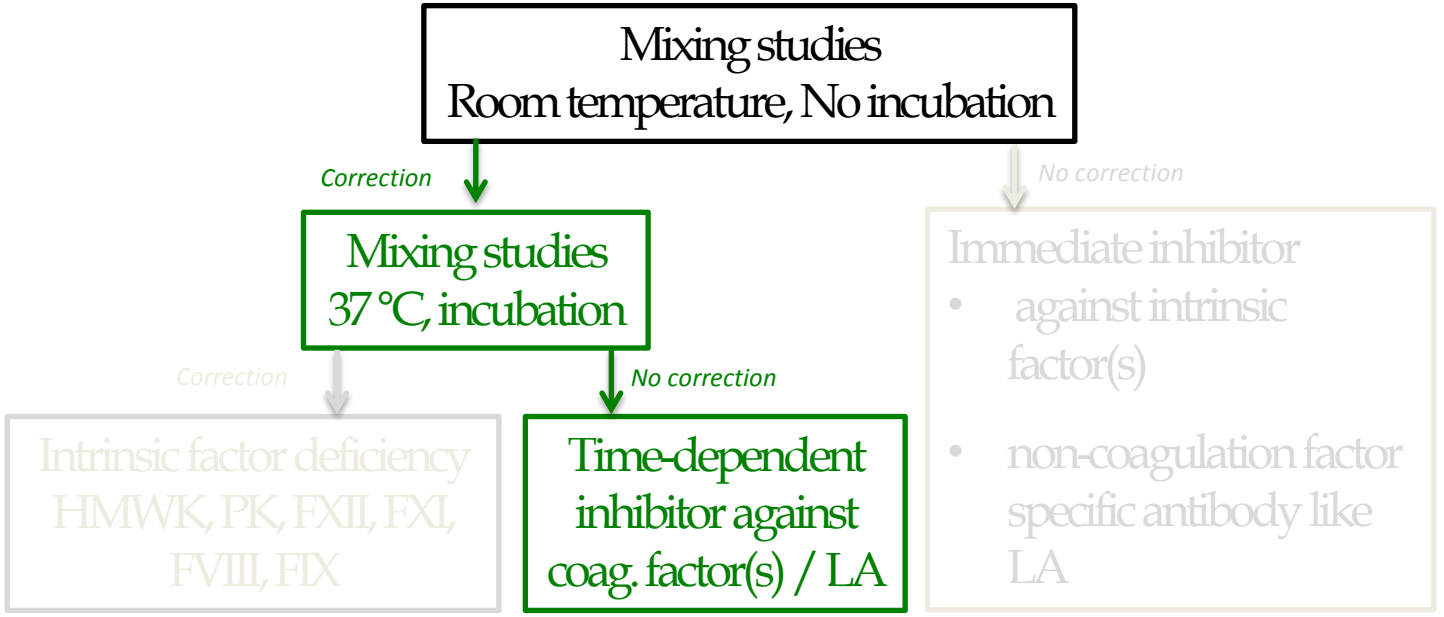
Immediate inhibitor

- against intrinsic factor(s)
- non-coagulation factor specific antibody like LA

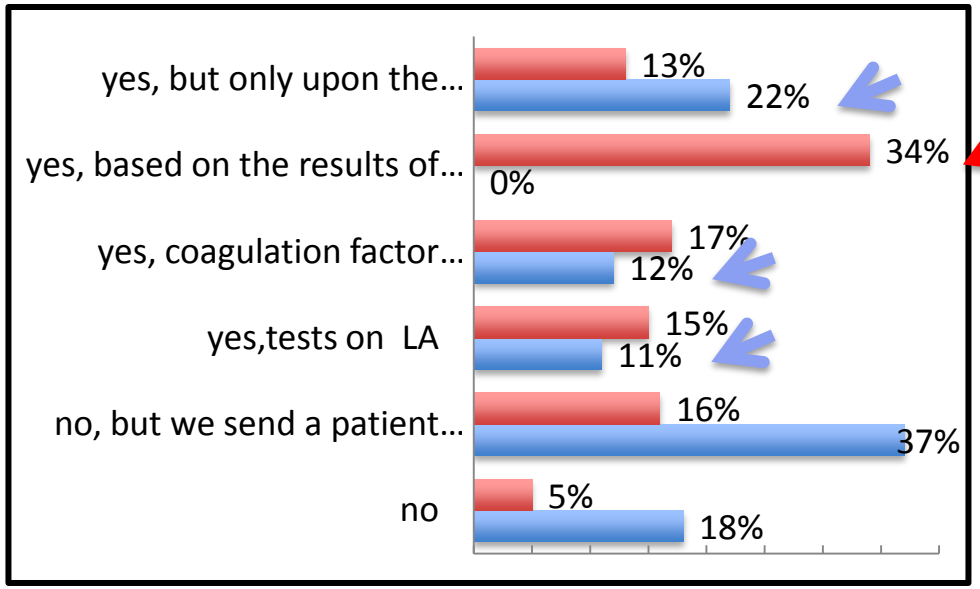
Interpretation of mixing study
Scenario C: time-dependent inhibitor

APTT 1:1 mixture of patient and pooled plasma	33 sec
APTT measured on pooled plasma	32 sec
APTT 1:1 mixture of patient and pooled plasma at 37°C	59 sec
APTT measured on pooled plasma at 37°C	35 sec





Further investigations



RANDOM TEST SELECTION

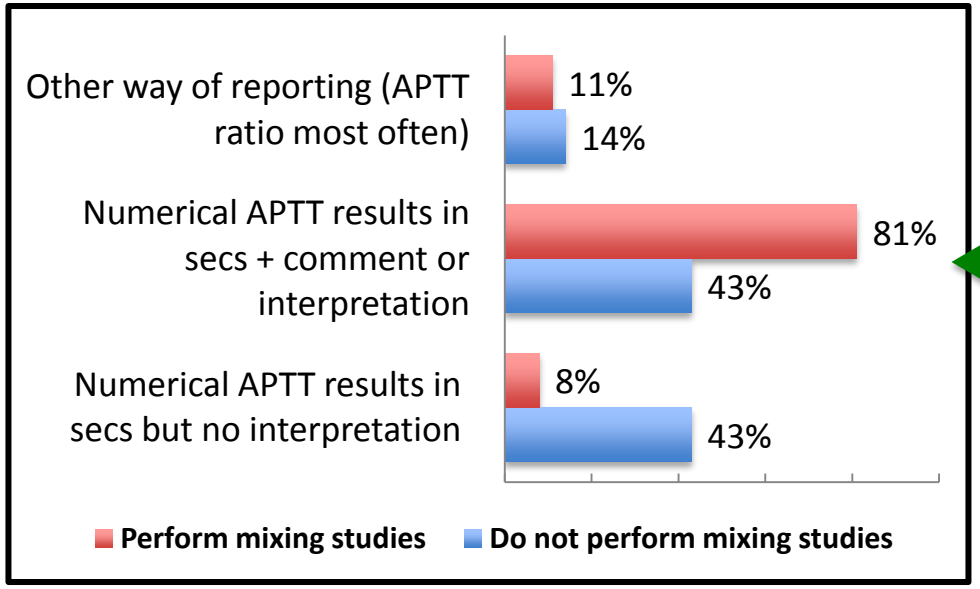
Only half of those laboratories that perform mixing studies and do further investigations would base them on the result of previous mixing studies.

23% of those laboratories that don't perform simple mixing study do either factor activity or LA tests or both as the next step in the investigations.

TEST SELECTION at the PHYSICIAN's REQUEST

22% non mixers 13% mixers

Reporting



81% of mixers and 43% of non-mixers states to provide comment or interpretations.

Validity of interpretations varies:

- Misinterpretations between mixers are quite frequent: 6-11%
- Non respondents ratio: 16-21%
- Partially correct: 47-57%

Summary

- The responses show considerable variation in management unexpected APTT prolongation in laboratories.
- 27% of laboratories do not perform mixing studies and 12% of laboratories do only at physician request passing this way the decision about the necessity of a reflex test in haemostasis investigation to the clinicians.
- Further investigations are often done only at the physician's request. Significant portion of laboratories seem to select test random, seem to have no clear protocol of step-by-step investigation of APTT prolongation.
- Majority of laboratories stated to provide interpretations in their report. Validity of interpretations of laboratory scenarios showed substantial variations.
- **Distribution of the survey's experience and guideline recommendations on APTT testing and interpretation can help to improve interpretative thinking and skill of participant laboratories.**