Standardization in Hemostasis Testing

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Reasons for Guidelines

• Provide recommendations to laboratories and staff as tools to optimize test performance and reporting

• Consistency in test performance

• Consistency in result reporting

• Harmonize results between laboratories
Types of Guidelines

• **Recommendations**
  • Suggestions to be followed
  • Based on evidence or tradition

• **Guidelines**
  • Strong suggestions, consensus based on expert opinion
  • Based on evidence or tradition
  • Must document why modifying guidelines

• **Standards**
  • Evidence based, and must be followed
## Sources of Guideline Development

<table>
<thead>
<tr>
<th>Source</th>
<th>Mechanism of Development</th>
<th>Type</th>
<th>Relevance</th>
<th>Geared Toward</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCSH</td>
<td>Expert Group</td>
<td>Guideline</td>
<td>Specialized</td>
<td>UK</td>
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<tr>
<td>CAP</td>
<td>Expert Individuals</td>
<td>Guideline</td>
<td>Sporadic</td>
<td>US</td>
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<td>CLSI</td>
<td>Consensus Panel</td>
<td>Guideline</td>
<td>Routine &amp; Specialized</td>
<td>World</td>
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<tr>
<td>ICSH</td>
<td>Expert Panel</td>
<td>Guideline</td>
<td>Specialized &amp; Routine</td>
<td>World</td>
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<tr>
<td>ISLH</td>
<td>Evidence Based (?)</td>
<td>Guideline</td>
<td>Specialized (?)</td>
<td>World</td>
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<td>ISO</td>
<td>Expert Panel</td>
<td>Guideline</td>
<td>Broad-based</td>
<td>World</td>
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<tr>
<td>ISTH-SSC</td>
<td>Expert Group</td>
<td>Guideline</td>
<td>Sporadic</td>
<td>World</td>
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<tr>
<td>WHO</td>
<td>Expert Group</td>
<td>Guideline</td>
<td>Broad-based</td>
<td>World</td>
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</table>

Edited from Richard Marlar
Issues associated with Hemostasis Guidelines

• Too few and too many
  • Too few: less than half of the coagulation testing has relevant guidelines
  • Too many: multiple organizations with guideline on same topic (e.g. lupus anticoagulant testing), without total harmonization

• How long are they valid?
  • Dynamic field that is changing
  • Length of document approvals

• Accessibility
  • No-charge (journals, only if open access)
  • Some must be purchased

• No complete list of available guidelines readily available (NASCOLA considering)
Issues associated with Hemostasis Guidelines

• Clinical and Laboratory Standards Institute
  • Multiple disciplines (e.g. chemistry, microbiology), multiple platforms (standards, guidelines, companion diagnostics), test, methods, administrative and instrumentation evaluation documents
  • Historical resource for hemostasis guidelines
    • Hemostasis guidelines: $140.00 each if non-member
• Changes to CLSI process:
  • Proposed documents no longer decided at the area committee level
  • Maximum number of documents per area committee per year, including revisions, is 3
  • Area committee for Hematology, now includes immunology in addition to hemostasis
  • Decision for proposed guideline heavily dependent upon proposed sales.
  • Takes 1.5 – 2+ years from proposal to publication
CLSI Hemostasis guidelines: for purchase

- H47-A2 One-Stage Prothrombin Time (PT) Test and Activated Partial Thromboplastin Time (APTT) Test, 2nd Edition 05/30/2008
- H48-Ed2 Determination of Coagulation Factor Activities Using the One-Stage Clotting Assay, 2nd Edition 03/30/2016
- H54-A Procedures for Validation of INR and Local Calibration of PT/INR Systems, 1st Edition 08/19/2005
- H59-A Quantitative D-dimer for the Exclusion of Venous Thromboembolic Disease, 1st Edition 03/31/2011
- H60-A Laboratory Testing for the Lupus Anticoagulant, 1st Edition 04/04/2014
- CLSI H51-A Assays of von Willebrand Factor Antigen and Ristocetin Cofactor Activity 09/01/2002 (Revision prepared, completed, CLSI decision not to publish, not listed)
British Society for Haematology (BSH)

- Formerly British Committee for Standards in Haematology
- Open access guidelines (aka free)
- Some graded (evidence based)
- Some expert opinion
- Clinical management, diagnosis, or laboratory guidelines
- More recent than CLSI
BSH Hemostasis testing guidelines

• Issued: 01/06/2015 Guideline Diagnosis and Management of Von Willebrand Disease
• Issued: 21/08/2014 Guideline Diagnosis and Management of Rare Coagulation Disorders
• Issued: 06/08/2014 Guideline Measurement of Non-Coumarin Anticoagulants and Their Effects on Tests of Haemostasis
• Issued: 14/06/2014 Guideline Diagnosis and Management of Acquired Coagulation Factor Inhibitor
• Issued: 01/07/2013 Guideline Diagnosis and Treatment of Factor VIII and IX Inhibitors in Congenital Haemophilia
• Issued: 25/05/2012 Guideline Investigation and Management of Antiphospholipid Syndrome
• Issued: 08/02/2012 Guideline Laboratory Investigation of Heritable Disorders of Platelet Function
• Issued: 11/03/2010 Guideline Testing for Heritable Thrombophilia
• Issued: 26/07/2006 Guideline Fibrinogen Assays
# ISTH/SSC Hemostasis testing guidelines

<table>
<thead>
<tr>
<th>SSC Committee</th>
<th>Publication years</th>
<th>Potency standard</th>
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<tbody>
<tr>
<td>Biorheology Subcommittee</td>
<td>2011, 2014</td>
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<tr>
<td>Factor VIII, Factor IX, and Rare Coagulation Disorders Subcommittee</td>
<td>2011 - 2014</td>
<td>2011, 2012</td>
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<tr>
<td>Perinatal and Paediatric Haemostasis Subcommittee</td>
<td>2012</td>
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<tr>
<td>Subcommittee on Control of Anticoagulation</td>
<td>2011, 2012 (x2), 2013 (x2)</td>
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<tr>
<td>Subcommittee on Factor VIII, Factor IX and Rare Coagulation Disorders</td>
<td>2014</td>
<td>2014</td>
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<tr>
<td>Subcommittee on Factor XI and the Contact System</td>
<td>2011</td>
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<tr>
<td>Subcommittee on Fibrinolysis</td>
<td>2016, 2017</td>
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<tr>
<td>Subcommittee on Haemostasis and Malignancy</td>
<td>2014</td>
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<tr>
<td>Subcommittee on Lupus Anticoagulant/Phospholipid/Dependent Antibodies</td>
<td>2014</td>
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<tr>
<td>Subcommittee on Platelet Immunology</td>
<td>2012 (x2), 2015, 2016</td>
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<tr>
<td>Subcommittee on Platelet Physiology</td>
<td>2013, 2015</td>
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<td>Subcommittee on Platelet Physiology</td>
<td>2014</td>
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<tr>
<td>Working Group on Coagulation Standards</td>
<td>2011</td>
<td></td>
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Guidelines in Development

- CAP
  - Protein C
  - Protein S
  - Antithrombin
- CLSI
  - Preanalytical variables
  - Heparin Induced Thrombocytopenia
- ICSH
  - Preanalytical variables
  - Direct oral anticoagulants
  - Factor VIII and IX inhibitor assays
- ISO
  - Unknown
- ISLH
  - Considering participation
- ISTH-SSC
  - Protein C
  - Protein S
  - Antithrombin
  - Activated Protein C Resistance
- BCSH
  - Unknown
- WHO
  - Unknown
ICSH Guideline: Direct Oral Anticoagulants (DOAC) Measurements

AIM:

• Intended as laboratory guideline
  • Not intended for, or recommending, whether patients should get tested while on DOACs

• Evidence based (peer-reviewed publications) or expert opinion with consensus
ICSH DOAC Committee members

• Dot Adcock - USA
• Shannon Bates - Canada
• Jonathan Douxfils - Belgium
• Robert Gosselin – USA (Chair)
• Isabelle Gouin-Thibault - France
• Cecilia Guillermo - Uruguay
• Emmanuel Favalaro - Australia
• Steve Kitchen - United Kingdom
• Yohko Kawai - Japan
• Edie Lindhoff-Last – Germany

Over 100 DOAC related peer-reviewed publications by the committee members
ICSH DOAC guideline: Sections

Background and DOAC description

• Drug details (indication, dose, bioavailability, etc)
• Anti-factor IIa DOAC (Dabigatran)
• Anti-Factor Xa DOAC (Rivaroxaban, Apixaban, Edoxaban)
• General DOAC recommendations (proposed)
  • Time collections if routine
  • Trough time collection
  • Results of quantitative assays be reported in ng/mL
Sample requirements

• Recommendations (proposed)
  • 3.2% sodium citrate sample for clot based or chromogenic assays
  • 3.2% or serum samples for mass spectrometry assays
  • If testing for dabigatran cannot be completed within 2 hours of collection, freeze sample
  • If testing for anti-Xa DOACs cannot be completed within 8 hours of collection, freeze sample
  • Up to three freeze-thaw cycles acceptable
Qualitative assays for DOACs

- PT, APTT, Thrombin time, thromboelastography

Recommendations (proposed)
- PT and/or APTT not suitable to detect “on-therapy” DOAC presence
- PT and/or APTT cannot be used to quantify DOAC
- A normal TT excludes significant levels of dabigatran
- Thromboelastometry may not have suitable sensitivity to detect on-therapy DOAC presence
Mass Spectometry measurement of DOACs

• Recommendations (proposed)
  • LC-MS/MS should be considered the gold standard for DOAC testing
  • A suitable internal standard is mandatory
  • DOAC metabolites, that are pharmacologically active, should be reported (consensus)
  • Enrollment in External Quality Assurance (EQA) program is mandatory
Other methods for quantifying FIIa DOAC (dabigatran)

- Ecarin based methods
  - Ecarin clotting time, ecarin chromogenic assay – commercial or LDT)
- Chromogenic FIIa commercial kits
- Dilute thrombin time (commercial or LDT)
- Recommendations (proposed)
  - Drug calibrated DTT, ECA, ECT, and anti-FIIa chromogenic methods comparable to LC-MS/MS dabigatran measurements.
  - Proper validation of drug calibrated methods is required prior to clinical use of assay.
  - Appropriate internal Quality Control is required, and needs to be performed at least every 8-24 hours (as predicated by regional regulatory bodies) of DOAC testing.
  - Enrollment in External Quality Assurance (EQA) program for continuous QA is required.
Other methods for quantifying anti-FXa DOACs

- Use of drug calibrated Anti-FXa commercial kits
- Recommendations (proposed)
  - Drug calibrated anti-FXa methods have demonstrated acceptable comparison to LC-MS/MS.
  - Antithrombin supplement anti-FXa methods should not be used for DOAC assessment as these methods tend to overestimate drug concentration.
  - Proper validation of drug calibrated anti-FXa chromogenic methods is required prior to clinical use of assay.
  - Appropriate internal Quality Control is required, and to be performed at least every 8-24 hours (as predicated by regional regulatory bodies) of DOAC testing.
  - Enrollment in External Quality Assurance (EQA) program for continuous QA is required.
POCT and assays in development

Assay validation

• Validation samples

• Proposal for determining assay:
  • Precision studies: Inter and Intra assay requirements
  • Accuracy studies: minimum 20 samples
  • Stability studies: short term and long term storage if no published data available for laboratory method
  • Suitability: assessing matrix effect

ICSH DOAC guideline: Sections
External Quality Assessment

• Recommendations (proposed):
  • Each laboratory must enroll in an EQA program specific for the DOAC being measured.
  • EQA should be at a minimum two samples per dispatch, with at least two dispatches in a calendar year.
  • EQA material should be measured using LC-MS/MS as the gold standard method for DOAC quantitation.
  • Each laboratory must maintain at least 80% agreement within a calendar year for EQA programs.