PEARLS OF LABORATORY MEDICINE

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**TITLE: Direct Oral Anticoagulants: Impact & Interference of DOACs on coagulation testing**

**PRESENTER: Bob Gosselin**

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**Slide 1:**

Hello, my name is Bob Gosselin. I currently serve as a volunteer with UC Davis Thrombosis & Hemostasis Center after my retirement in 2017 from the university health system as the senior Clinical Laboratory Scientist in special coagulation. Welcome to this Pearl of Laboratory Medicine on "**Direct Oral Anticoagulants, Impact & Interference of DOACs on coagulation testing**." This program was created with Dr Dot Adcock, the Chief Medical Officer and senior vice president of LabCorp Diagnostics.

**Slide 2:**

This session is a joint effort between the American Association for Clinical Chemistry (AACC) and the North American Specialized Coagulation Laboratory Association (NASCOLA)

**Slide 3:**

In the previous sessions we provided an overview of DOACs which include dabigatran, a direct thrombin inhibitor, and the direct anti- Xa DOACs which include apixaban, betrixaban, edoxaban and rivaroxaban. We previously described their performance characteristics, including pharmacodynamics and pharmacokinetics using screening or specific coagulation assays.

The new consideration:
As DOACs target specific activated factors, the impact of these drugs on other coagulation assays are likely leading to potential erroneous interpretation and mismanagement.

**Slide 4:**

As we previously demonstrated that DOACs may affect clot-based assays such as the PT and APTT, but will these observations translate to other clot-based coagulation assays? Will DOACs also affect non-clot-based assays, such as immunoassays? As trough levels represent the lowest DOAC concentration in a patient, will trough collections mitigate any DOAC interference? Lastly, are there are any alternative strategies for testing in the presence of DOACs?

**Slide 5:**

This slide represents a review of clot-based coagulation testing, includes the screening tests PT/INR and APTT, factor assays, thrombophilia testing and other routine or esoteric assays. In prior Pearls, we have extensively described the DOAC effect on screening tests such as PT and APTT. As DOACs are inhibitors of activated coagulation factors, PT and/or APTT mixing studies may mimic an inhibitor pattern.
In this session we will be describing a general overview of the effect of DOACs on more esoteric assays.

**Slide 6:**

The one-stage assay is the most common method used for determining factor activity. Factor deficient plasma is added to diluted patient sample, then either a PT or APTT is run to obtain a corresponding clot time.

The clot time is inversely proportional to factor activity, so the shorter the clotting time, the higher the factor activity. A calibration curve with a standard of known factor activity will provide a relationship between factor activity and clot times. The factor activity of an unknown or patient sample is extrapolated from the calibration curve based on the clot times obtained.

As DOACs may increase PT or APTT clot times, this will result in factitiously low factor activity as noted with increasing concentrations of dabigatran and reported factor II and factor VIII activity.

So caution must be exercised when performing factor activity testing in DOAC treated patients as this may lead to misdiagnosis and potential mismanagement.

This caution also applies to clot-based inhibitor assays, such as the Bethesda assay, which may suggest the presence of a factor inhibitor.

**Slide 7:**

Fibrinogen and thrombin time are similar assays in that thrombin is added to patient sample, with the difference between the two assays is the sample preparation and thrombin concentration.

As thrombin is the test reagent, dabigatran, the direct thrombin inhibitor will prolong the clotting time and result in falsely depressed fibrinogen results and prolonged thrombin time.
Direct factor Xa DOACs will not affect either test. The factitiously low fibrinogen levels are dependent on the dabigatran concentration and the testing protocol including sample dilution and thrombin concentration.

**Slide 8:**

Clot base protein C uses a snake venom, Protac, from the copperhead snake Agkistrodon contortrix. Protac will activate the protein C in the sample, creating activated protein C which inhibits activated factors VIII and V. As activated V and VIII are inhibited, the clotting time is prolonged. The prolongation is proportional to amount of protein C present in the plasma. . Protein C deficient plasma is added to test sample to isolate clot time prolongation is only due to protein C.

DOACs may increase the APTT clotting time, thus falsely elevating Protein C results.

There is a variable DOAC dose dependent effect on clottable protein C methods, resulting in factitiously elevated Protein C levels.

Alternatively, chromogenic protein C methods are not affected by either class of DOACs

As demonstrated no effect of either dabigatran or rivaroxaban on chromogenic protein C results.
While the clot-based PC assay described here is the most common, there are other reagent platforms using prothrombin time or Russell's Viper Venom time reagents. The DOAC effect on these methods have not been adequately described but may be vetted with external quality assurance samples provided by CAP and ECAT. Immunologic methods for protein C are not affected by DOAC presence.

**Slide 9:**

Protein S is a co-factor to activated protein C, and clot base protein S testing uses the principle for testing. Protein S deficient plasma is added to test sample to isolate clot time prolongation is only due to protein S. Activated protein C is then added to the sample, creating activated protein S, which will inhibit factor Va which is either provided as a reagent source or through snake venom activation of factor Xa, which will subsequently activate factor V. The factor Va inhibition by protein S is proportional to the prolongation of the clotting time.

DOACs will increase the clotting time,

thus falsely increase reportable protein S activity using clot based methods. As noted with protein C, there is variation between DOACs, but all will potentially yield falsely elevated protein S activity.

Alternatively, free protein S using latex immunoassay methods is not affected by DOACs,

As demonstrated with dabigatran in this figure.
While the clot-based functional PS assay described here is the most common, there are other reagent platforms using prothrombin time or Russell's Viper Venom time reagents. As with alternative PC assays, the DOAC effect on these methods DOACs have not been described in detail but may be vetted in the future with external quality assurance programs such as CAP and ECAT.

**Slide 10:**

Activated protein C resistance is a screening method for factor V gene mutations that result in resistance to neutralization of factor Va by APC. This assay is a modified APTT, with one step using standard APTT method, the second step using a calcium reagent supplemented with activated protein C.

As DOACs will increase clotting times of the APTT, false negative ratios may be obtained as noted in samples with increasing concentration of DOACs

Additionally, false positive APCR results have been reported.
Alternatively, genetic testing for factor V Leiden can be performed, but this may miss unusual activated protein C mutations, such as V Cambridge or Hong Kong.

**Slide 11:**

For lupus anticoagulant testing, although there are several methods available, there are two primary testing principles: the dilute Russell's Viper Venom time or DRVVT and the hexagonal phase method. For the DRVVT, the venom from Russell's viper activates factor X in a low phospholipid concentration reagent. If prolonged, a repeat DRVVT in a high phospholipid concentration reagent is tested, and the ratio between the two results would suggest the presence or absence of a lupus anticoagulant. With hexagonal phase method, a modified APTT with and without phosphatidylethanolamine is measured and if the result difference between the two tests exceed 8 seconds, then a lupus anticoagulant is present.

With DRVVT methods, DOACs may increase clotting times, thus falsely indicating a lupus anticoagulant.

Which increases with increasing DOAC concentration.

Anti-Xa DOACs do not interfere with hexagonal phase testing, but dabigatran has been demonstrated to cause false positive results at higher concentrations

As noted in this figure, with exceeding the 8 second difference threshold for hexagonal phase testing at approximately 200ng/mL of dabigatran.

**Slide 12:**

As a note, because of the linear relationship seen between the DRVVT test and DOAC concentration, this method has been proposed as a means of quantifying DOACs.
The concern however is in the lower concentration of DOAC, in this example being rivaroxaban,
And perhaps the lower limit of quantitation may not be sufficiently adequate.

**Slide 13:**

Chromogenic methods are typically two stage assays, where the sample is mixed with activator in the first stage, then the addition of a specific substrate. This cartoon depicts the antithrombin assay, where the antithrombin in patient sample complexes with excess factor Xa in the presence of heparin, resulting in a bound complex plus residualy factor Xa. A specific substrate is added which binds to the residual factor Xa, which results in peptide cleavage and yellow color production. For this particular chromogenic assay the yellow color production is inversely proportional to antithrombin present.

Of particular note for DOACs, is some antithrombin methods use excess thrombin instead of factor Xa, but the principle still the same with excess thrombin cleaving specific substrate resulting in yellow color production.

**Slide 14:**

In this figure, the effect of anti-Xa DOACs demonstrate the falsely increased reported antithrombin activity within increasing drug concentration using the factor Xa reagent method. Note that dabigatran does not affect the Factor Xa method. If the reagent were, thrombin based, then the representation would be reversed where the dabigatran samples will show falsely increased levels with increasing drug concentration, and no effect by anti- Xa DOACs.

**Slide 15:**

This table lists the common chromogenic assays that are avaible. There is no uniform effect, so clinicians and laboratory personnel should be aware of potential DOAC interference prior to result reporting. However, as laboratorians tend to have the greater knowledge about technical issues related to testing, they should take the lead role in

providing information to clinicians about DOAC interference on coagulation assays and provide recommendations for alternative tests or methods.

**Slide 16:**

The test methods that are not affected by DOACs include leteximmunoassays, ELISA based methods, agglutination methods, or most platelet function testing.

Esoteric tests that may be affected by DOAC presence include thrombin based platelet aggregation, thrombin activatable fibrinolysis inhibitor and these drugs may alter assays that assess fibrinolysis.

**Slide 17:**

In a previous publication we demonstrated the estimated DOAC concentration that resulted in a >15% difference from baseline. The columns represent the specific DOACs, with expected trough levels.
For dabigatran x7
For apixaban x3
For betrixaban x2
For edoxaban x2
For rivaroxaban x 6
Note that these assays were affected by low DOAC concentrations that may be expected for trough concentrations. As such, merely collecting trough samples may not be sufficient to avoid factitious results due to DOAC interferenceThe LA testing reported here is for Dilute Russell's Viper Venom method, with the final ratio reporting be less affected by DOAC presence than the screening and confirmation methods. These data reflect method and reagent dependence.

**Slide 18:**

Minimizing DOAC interference…
Select tests that are not affected by DOACs would be the optimal solution. There are in-vitro neutralizing agents, such as activated charcoal and filters that are commercially available.

However notes of caution:
None of these neutralizing products are currently FDA approved

These products are not interchangeable

Some of these products induce some degree of coagulation

There is variable degree of plasma recovery after using these neutralizing products

**Slide 19:**

In summary about DOACs and interference with coagulation testing
Are clot-based methods affected by DOACs?

This is mostly a true statement

Are non-clot based methods not affected by DOAC presence?

This also is mostly true, but chromogenic methods may be affected depending on substrates.

Will collection of trough samples be sufficient to mitigate any DOAC test interference?

This is cautiously false, as this will be method dependent.
Alternative strategies for testing while on DOACs.

Consider alternative methods or DOAC neutralizing agents.

**Slide 20: References**

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### Slide 21: Disclosures

Dorothy Adcock has received honoraria from Siemens Healthcare Diagnostics and is a consultant to Instrumentation Laboratory.
Robert Gosselin has provided expert testimony for dabigatran and rivaroxaban testing, has received honoraria from Siemens Healthcare Diagnostics, Machaon Laboratories and Diagnostica Stago, and serves as a consultant for Diagnostic Grifols and UniQure, and advisory board member for BioMarin.

### Slide 22: Thank You from www.TraineeCouncil.org

Thank you for joining me on this Pearl of Laboratory Medicine on "**Impact & Interference of DOACs on coagulation testing**".