Diagnosis and classification of von Willebrand Disease (VWD)

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Talk outline: aim to review

- Laboratory tests used in the diagnosis of VWD.
- Issues related to these tests
- Revised classification scheme for VWD
- Potential utility of additional test processes to help identify and functionally characterise VWD.

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Von Willebrand Disease (VWD)

- Most common inherited bleeding disorder.
- Arises from deficiencies or defects in von Willebrand factor (VWF).
- VWF has two primary functions/roles:
  - 'carries' FVIII, and protects/stabilises FVIII:C function.
  - permits adhesion of platelets to sites of vascular damage.
- VWF is a multimeric protein
  - low to high molecular weight or 'small' to 'large' size
  - higher MW = greatest adhesive function.

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Diagnosis of VWD

- *Clinical Review:*
  - Bleeding history (spontaneous/surgery)
  - Family history (siblings/parents/grandparents)
  - Age, gender, recent medication history, blood group
  - Pregnancy, oestrogen therapy, menstrual cycle
  - Physical examination ('bruising')
- *Laboratory Investigation:* Many different possible approaches with differing 'efficacy'.

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Von Willebrand Disorder (VWD): Classification

- 'Recently' revised & published by ISTH VWD SSC (Sadler et al, J Thromb Haemost, 2006; 4: 2103-14).
- Previous 'update' was 1994.
- Basic 2006 classification largely unchanged from 1994 except for some key 'adjustments'.

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Pre-1994: At Least 25 Types of VWD in 1992

I, I-1, I-2, I-3, I platelet normal, I platelet low, I platelet discordant, IA, IB, IC, ID, I New York

IIA, IIA-1, IIA-2, IIA-3, IIB, IIC, IID, IIE, IIF, IIG, IIH, II-I, Malmö

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B, Vicenza, Normandy

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Main emphasis for classification in 1994:

⇒Must have clinical utility

⇒Simple, with a minimum number of categories

⇒Dependent mainly on common tests

⇒Emphasize concepts (definitions) over specific tests (implementation)

⇒VWD is caused by mutations in the *VWF* gene

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Main impetus to changes to classification 1994 - 2006:

- Many basic and clinical studies including molecular Type 1 & 2 VWD, and synthesis / clearance (including ADAMTS-13).
- Intent is to preserve simplicity and clinical utility.
- Intent is to be independent of laboratory tests because these may be refined with time.

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Von Willebrand Disorder (VWD): 'Primary' Classification*

- *Type 1:* Partial quantitative deficiency of VWF (reduced levels of functionally normal VWF).
- *Type 3:* Virtually complete deficiency of VWF (VWF essentially 'absent').
- *Type 2:* Qualitative VWF defects (absolute levels of VWF low or normal, but VWF 'function' diminished).
  - 2A, 2B, 2M, 2N

* Concepts largely unchanged from 1994 as general classification except in some key points

Sadler et al, J Thromb Haemost, 2006; 4: 2103-14

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VWD: Secondary Classification: Type 2 (qualitative) variants*

- *Type 2A:* decreased VWF-dependent platelet adhesion and a selective deficiency of HMW VWF multimers (in plasma).
- *Type 2B:* Increased affinity (of VWF) for platelet gp-Ib (leading to loss of HMW-VWF multimers in plasma).
- *Type 2M:* Decreased VWF-dependent platelet adhesion without a selective deficiency of HMW VWF multimers (in plasma).
- *Type 2N:* Markedly decreased binding affinity (of VWF) for factor VIII (leading to relative reduction in plasma FVIII:C).

* minor textual refinement from 1994 but broadly same groups; 'removed' concept of primary & secondary classification (now 6 types, not 3 types and 4x type 2 'subtypes')

Sadler et al, J Thromb Haemost, 2006; 4: 2103-14

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Main changes to classification 1994 - 2006:

- Remove stipulation that VWD caused by VWF mutations (in practice not usually investigated or achievable and ignores non-VWF gene related events).
- Expand the definition of VWD type 1
  - No significant decrease in the proportion of HMW multimers
  - Circulating VWF may or may not contain mutant subunits
  - Thus, now includes some subtypes previously in Type 2; eg Vicenza.
  - Problem - some labs now identify 2M VWD cases as type 1 VWD
- Inclusion of the potential utility of functional VWF/VWF:Ag ratios to identify functional discordance (& hence Type 2 VWD).

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Identification/discrimination of VWD types:

Importance:

- Presenting biological activity (ie quantity & quality) of VWF determines the haemorrhagic risk.
- Clinical/therapeutic management differs accordingly (DDAVP vs factor concentrates).

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DDAVP for VWD:

VWD Type	*Effective Response*
Type 1	Usually
Type 2A	Sometimes
Type 2B	Contraindicated?
Type 2M	Sometimes
Type 2N	Sometimes
Type 3	Rarely

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VWD Diagnosis:
Laboratory Assessment

- **Screening tests (APTT, [PT], FBC/platelet count/Hct, [SBT], PFA-100, [PFS]).**
- **Primary 'Diagnostic' assays (FVIII:C, VWF:Ag, VWF:CB, VWF: RCo).**
- **Secondary 'Confirmatory/VWD-subtype assisting' assays (2A, 2B, 2M - RIPA, VWF:Multimers; 2N - VWF:FVIII binding assay).**
- **Supplementary assays (propeptide, VWF binding, etc)**

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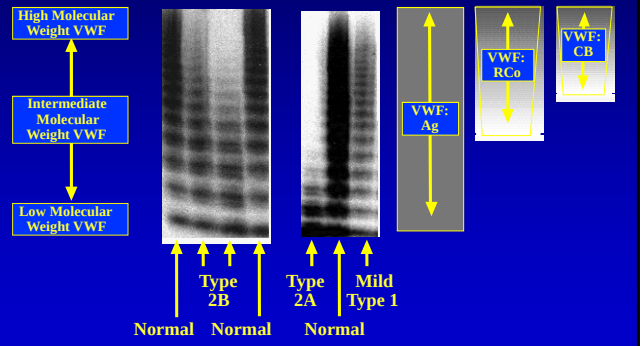
Detection of VWD:

'Relationship' between VWF:Multimers,

VWF:Ag, VWF:RCo & VWF:CB



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Detection of Type 1 VWD: *Laboratory process:*

- **Type 1 VWD - low but similar or 'concordant' VWF test result patterns:**
  - **ie non-functional [VWF:Ag] and functional [VWF:CB, VWF:RCo] are all low ('<50%') but show similar values**
  - **ie ratios ~ 1.0 for any VWF test comparison**
  - **take care with ratios if VWF <15% because of assay limitations at low VWF levels (especially if RCo value is the denominator).**
  - **Assay variability & LOD issues**

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Detection of Type 3 VWD: *Laboratory process:*

- Type 3 VWD - very low or absent VWF test result patterns (again similar or 'concordant'):
  - ie non-functional [VWF:Ag] and functional [VWF:CB, VWF:RCo] are all absent or very low but similar values.
  - (NB: don't attempt ratio estimations for VWF <15%, so don't attempt ratio estimations for Type 3 VWD).
  - LOD issues

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Detection of Type 2 VWD: *Laboratory process:*

- Type 2 VWD - 'discordant' test result patterns:
  - Type 2A, 2B and 2M: discordance between VWF results (ie non-functional [VWF:Ag] vs functional [VWF:CB, VWF:RCo]).
  - Type 2N: discordance between VWF and FVIII.
  - Assay variability, assay sensitivity to HMW VWF & LOD issues

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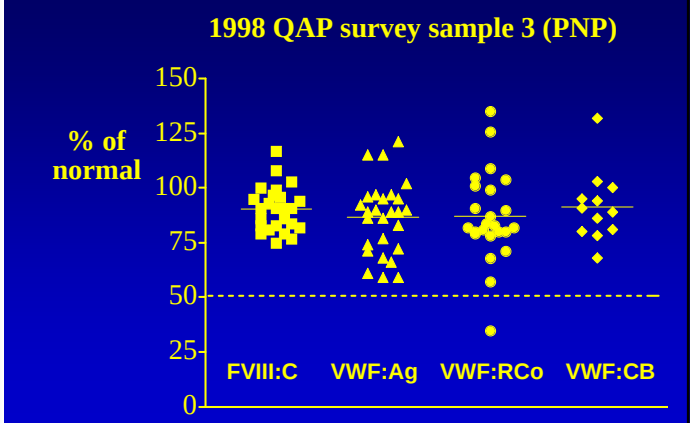
Assay variability -



Favaloro et al, Thromb Haemost, 1999; 82: 1276-82.

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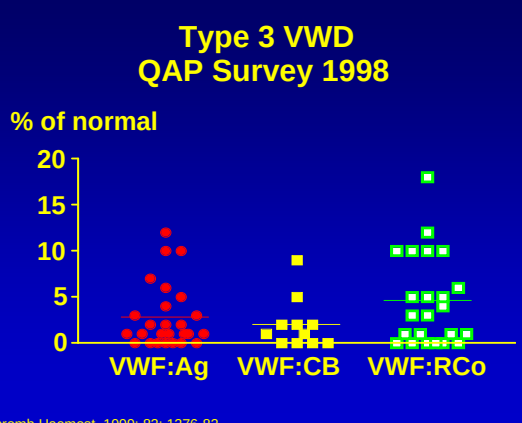
Assay limit of detection (LOD) / sensitivity -



Favaloro et al, Thromb Haemost, 1999; 82: 1276-82.

Favaloro et al, Clin Lab Sci, 2008; 21: 178-185

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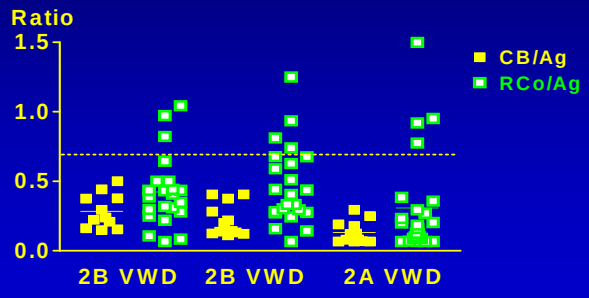
Assay sensitivity to loss of HMW VWF -



Modified from Favaloro et al, Thromb Haemost, 1999; 82: 1276-82.

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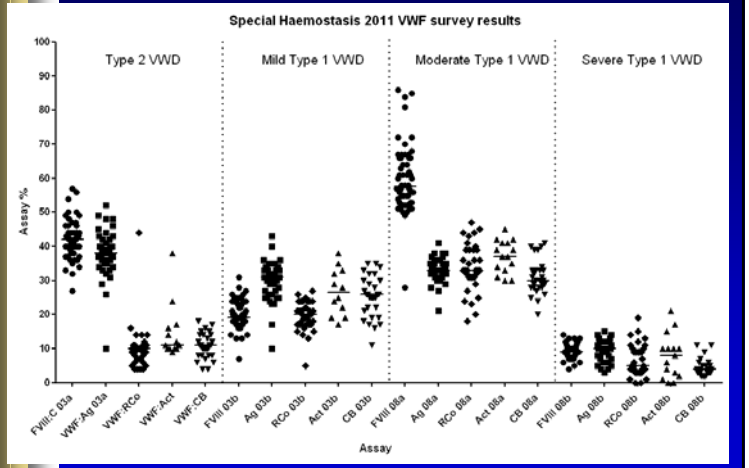
Recent data RCPA QAP -



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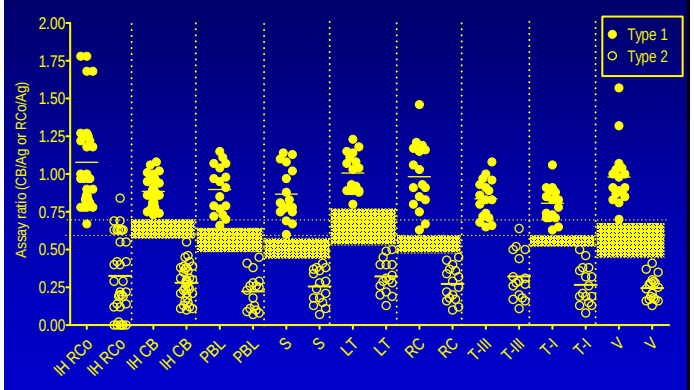
Assay sensitivity to loss of HMW VWF -



Favaloro et al, Thromb Haemost, 2010; 104: 1009-21.

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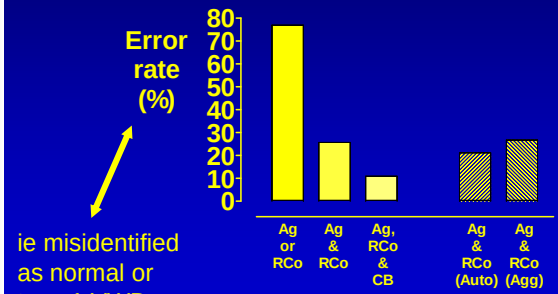
RCPA QAP data 2006
VWF functional assays compared:
Type 2B VWD
(FVIII:C plus…)



ie misidentified as normal or type 1 VWD

Favaloro et al, Thromb Haemost, 2007; 98:346-358 .

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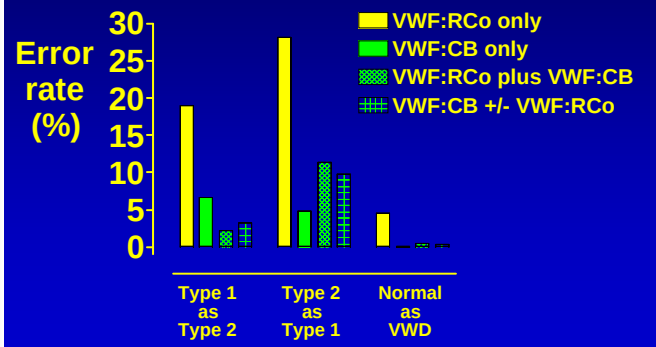
RCPA QAP data 1998-2005
VWF functional assays compared:
(VWF:Ag plus FVIII:C plus…)



Favaloro et al, Semin Thromb Hemost, 2006; 32: 505-13.

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PFA-100

- Whole blood - 5 min platelet function test.
- High shear stress flow system ('pseudo-physiological').
- Assesses cessation of blood flow (closure time = CT) after blood makes contact with a membrane coated with platelet agonist immobilised on a membrane in a disposable cartridge.
- Two cartridge types (C/ADP & C/Epi) with differing sensitivities.

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PFA-100

- Very sensitive to presence or absence of plasma VWF
- Therefore, sensitive to VWD
- 100% sensitive to Types 2A, 2M, 2B, and 3 VWD.
- ~80% sensitive to Type 1 VWD, with increasing sensitivity according to reduction in plasma VWF

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PFA-100

- Reasonable negative predictor
  - Normal PFA = VWD very unlikely
  - Normal PFA = VWD types 3, 2A, 2M, 2B can probably be excluded (>99% 'certain')
- Prolonged PFA-100 CT 'less' informative
  - May be VWD
  - May be platelet dysfunction,
  - May be low platelet count, medication (eg aspirin, NSAIDs) or low haematocrit
- Either case, further testing required, because
  - PFA is not a diagnostic test
  - Normal PFA can still occur with clinical bleeding, so need specific testing
  - Abnormal PFA does not define any specific disorder, so need specific testing

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DDAVP trials/challenge:

- Assays used to help identify or diagnose VWD can also be used to monitor therapy (eg DDAVP trial, VWF concentrate trial).
- VWF:Ag, VWF:RCo, VWF:CB, FVIII:C, PFA-100, platelet count (FBC).
- DDAVP trials can also assist to help identify and functionally characterize VWD.

References:
1. Favaloro et al. Am J Hematol, 1994; 45:205-11.
2. Favaloro et al. Haemophilia, 2001; 7:180-9.
3. Favaloro EJ. Semin Thromb Hemost, 2006; 32: 566-76.
4. Favaloro et al. Thromb Res, 2009; 123:862-8.
5. Favaloro EJ. Semin Thromb Hemost, 2009; 35:60-75

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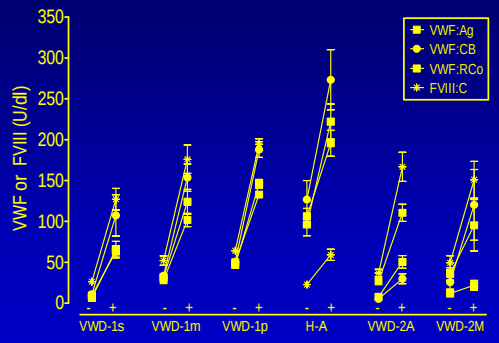
DDAVP trial as an aid to diagnosis



Favaloro EJ, et al. Thromb Res, 2009; 123: 862-868
Favaloro EJ, et al. Blood Coag Fibrinolysis, 2009; 20:475–483
Favaloro EJ. Semin Thromb Hemost 2009, 35: 60–75

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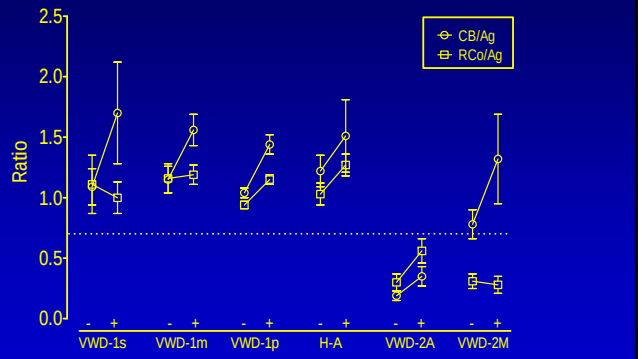
DDAVP trial as an aid to diagnosis



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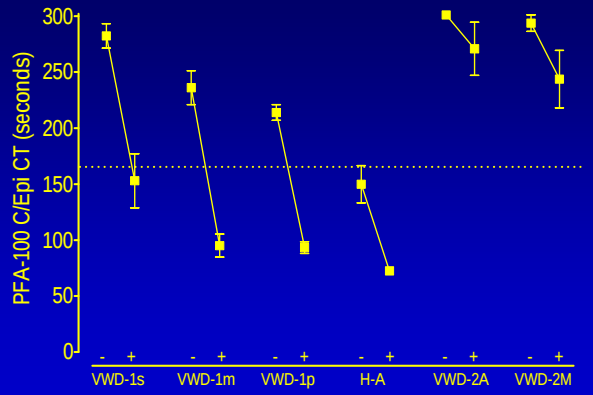
Favaloro EJ, et al. Thromb Res, 2009; 123: 862-868
Favaloro EJ, et al. Blood Coag Fibrinolysis, 2009; 20:475–483
Favaloro EJ. Semin Thromb Hemost 2009, 35: 60–75

DDAVP trial as an aid to diagnosis



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Favaloro EJ, et al. Thromb Res, 2009; 123: 862-868
Favaloro EJ, et al. Blood Coag Fibrinolysis, 2009; 20:475–483
Favaloro EJ. Semin Thromb Hemost 2009, 35: 60–75

Recent VWF:RCo improvements:

- ‘Low curve’ concept (factor assays) methodology
  - Better LOD (<5% rather than 10-20%)
  - Better discrimination of type 1 vs 3 VWD
  - Better discrimination of type 1 vs 2 VWD

References:
1. Hillarp A, et al. J Thromb Haemost 2010; 8: 2216–23.
2. Favaloro EJ, et al. J Thromb Haemost 2010; 8: 2842–4.

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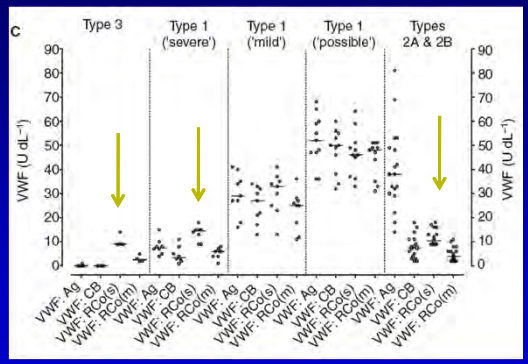
Better LOD



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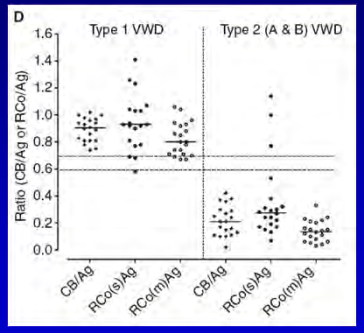
Better assay ratios



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VWF 'Activity' assays:

- Monoclonal antibody based assays are not the same as VWF:RCo
  - No ristocetin used
  - Monoclonal antibody based assays
  - Non- VWD, Type 1 VWD and 3 VWD will give similar results
  - Type 2A, 2B and 2M may not
- New 'activity' assays based on recombinant gp1b

References:
1. Chen D, et al. J Thromb Haemost. 2011;9:1993-2002
2. Rodgers S, et al. Sem Thromb Haemost;37:535-41

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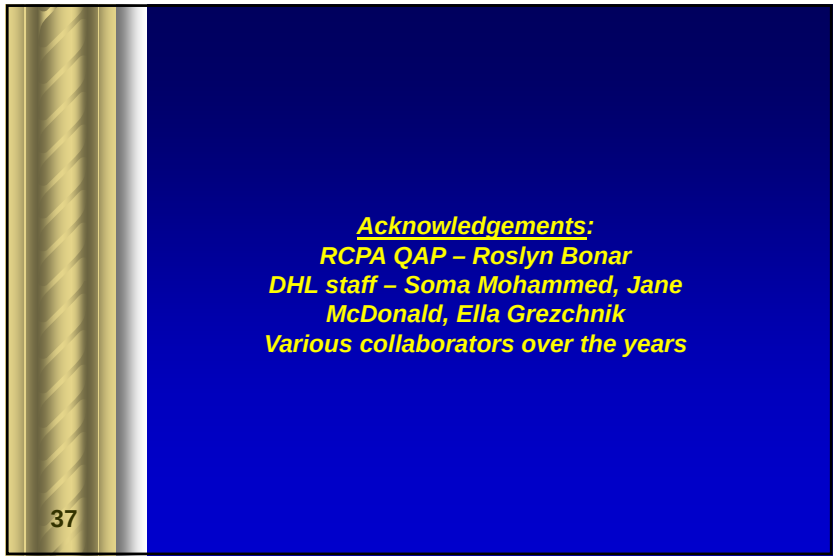
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