Resultaten DBS ringonderzoek immunosuppressiva

Hoe presteren DBS analysemethoden in praktijk?

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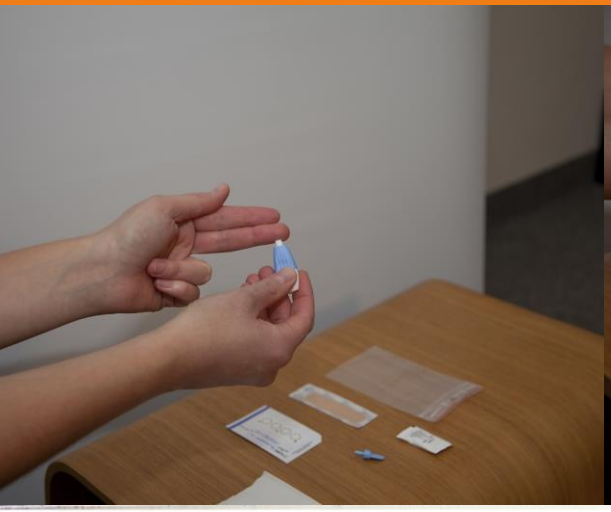
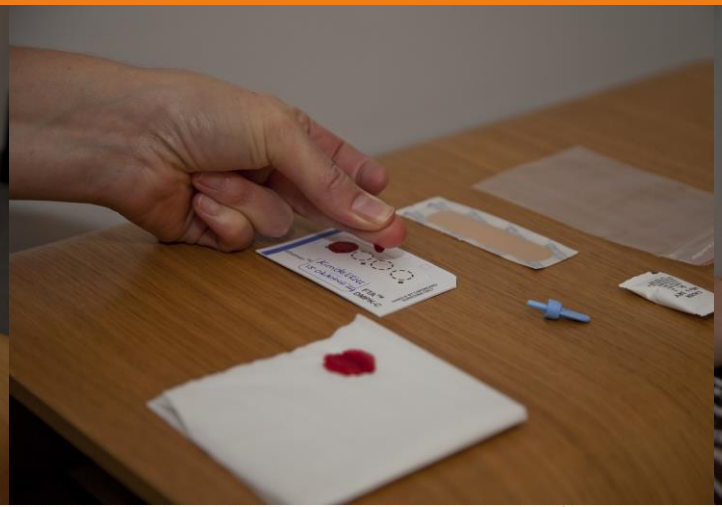
KKGT discussiedag, 02 april 2019





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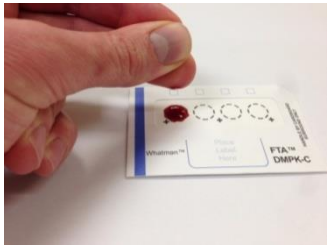

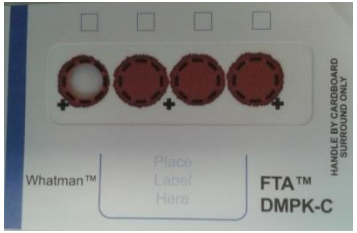

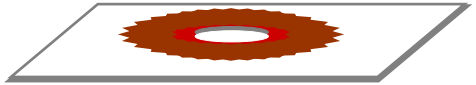

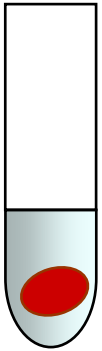

RESULT

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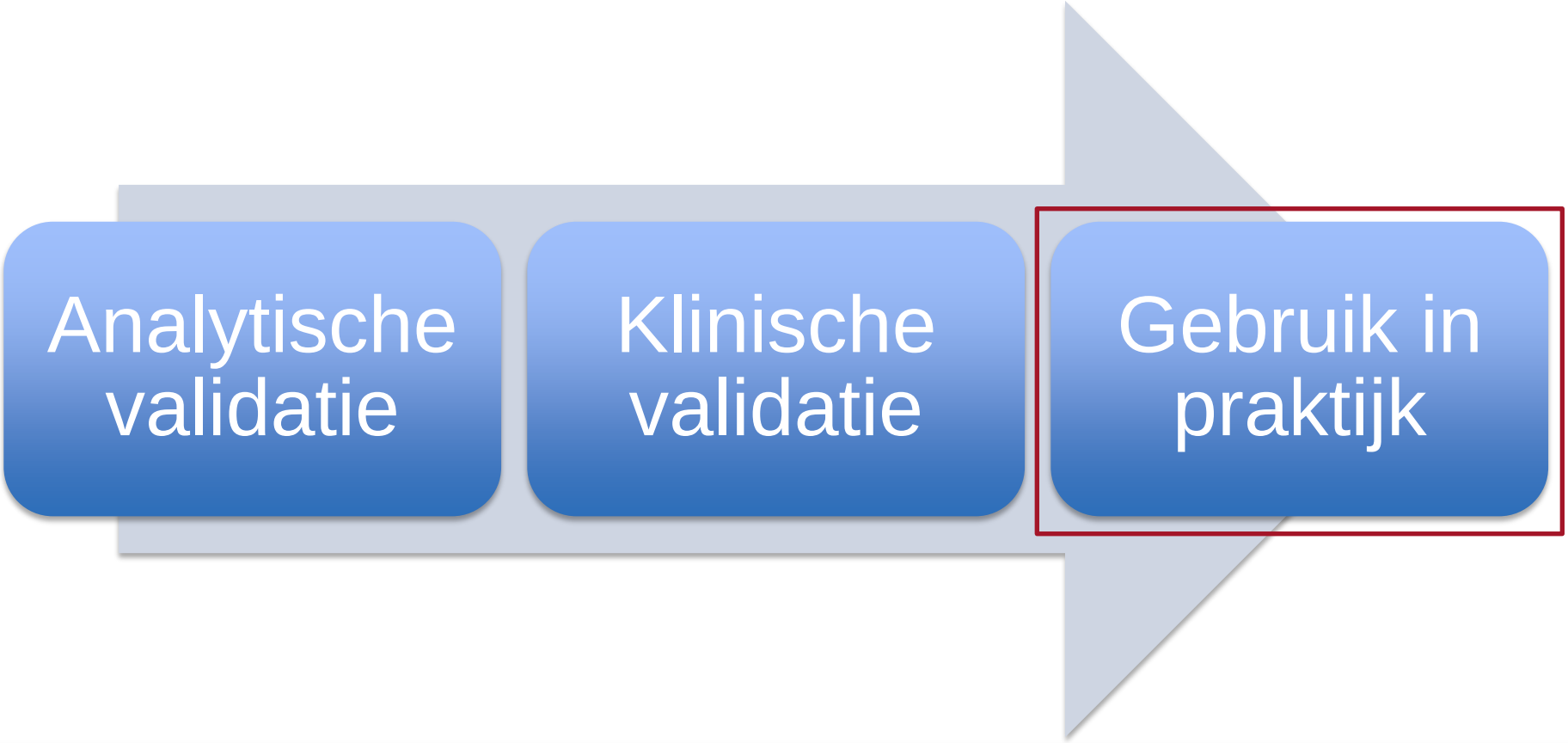

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Overzicht

- Waarom DBS ringonderzoek?
- Opzet ringonderzoek
- Resultaten
- Mogelijke verklaringen
- Gemaakte afspraken
- Conclusies

Waarom ringonderzoek?

- ISO: “all medical laboratories are required to participate in inter-laboratory comparison to ensure quality, comparability and acceptability of analytical results”

- 6/7 deelnemers gebruiken DBS om patiënten te behandelen.

- **Er is op dit moment geen enkele vorm van ringonderzoek of externe kwaliteitscontrole voor TDM DBS methoden**

Opzet

- 7 deelnemers (6 NL, 1 USA)
- Zo veel mogelijk gelijkend op de klinische situatie
- Gespiked volbloed gespot op DBS kaartjes (Whatmann DMPK-C of 903)
- Ronde 1: DBS + vragenlijst
- Ronde 2: DBS + volbloed + QC & standaarden

Table 1 – Absolute reported values by all labs – round 1

Lab	2	3	4	5	6	7	Range	Spike value
C1: Cyclosporin A in (µg/L)							38 - 55	50
C2: Cyclosporin A in (µg/L)							139 - 254	200
C3: Cyclosporin A in (µg/L)							402 - 898	700
T1: Tacrolimus in (µg/L)							2.3 - 3.7	3
T2: Tacrolimus in (µg/L)							14.0 - 20.6	18
T3: Tacrolimus in (µg/L)							29.6 - 47.8	40
Creatinine in µmol/L								
Hematocrit (v/v)								0.41

Table 2 – Percentage of reported values compared to the spike value – round 1

Lab	2	3	4	5	6	7	Mean (%)	Bias (%)	Presicion (rsd)(%)	Range (%)	Spike value (µg/L)
C1: Cyclosporin A (%)							90.3	-9.7	19.2	75.3 - 110.0	**50**
C2: Cyclosporin A (%)							94.3	-5.7	28.8	69.5 - 127.0	**200**
C3: Cyclosporin A (%)							89.5	-10.5	36.2	57.4 - 128.3	**700**
T1: Tacrolimus (%)							106.8	6.8	17.4	75.0 - 123.3	**3**
T2: Tacrolimus (%)							101.0	1.0	13.2	77.9 - 114.4	**18**
T3: Tacrolimus (%)							98.5	-1.5	18.2	74.0 - 119.5	**40**

	Within 15% of spiked value	
Yes	13	39.4%
No	20	60.6%
Total	33	100.0%

	Within 26.5% of spiked value	
Yes	27	81.8%
No	6	18.2%
Total	33	100.0%

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Table 3a – Percentage of reported values compared to the average – round 1

Lab	2	3	4	5	6	7	Mean (%)	Bias (%)	Presicion (rsd) (%)	Range (%)	Average (µg/L)
C1: Cyclosporin A (%)							100.0	0.0	19.2	83.4 - 121.9	**45.1**
C2: Cyclosporin A (%)							100.0	0.0	28.8	73.3 - 134.7	**188.5**
C3: Cyclosporin A (%)							100.0	0.0	36.2	64.2 - 143.3	**626.5**
T1: Tacrolimus (%)							100.0	0.0	17.4	70.2 - 115.4	**3.2**
T2: Tacrolimus (%)							100.0	0.0	13.2	77.1 - 113.3	**18.2**
T3: Tacrolimus (%)							100.0	0.0	18.2	75.1 - 121.3	**39.4**

	Within 15% of spike value	
Yes	16	48.5%
No	17	51.5%
Total	33	100.0%

	Within 26.5% of spiked value	
Yes	24	72.7%
No	9	27.3%
Total	33	100,0%

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Ronde 2

- Volbloed + DBS + patiëntmonster
- Stabiliteit volbloed
- ‘Spike’ waarden
- EDTA vs Citraat

Table 4 – Absolute reported values by all labs – Round 2

DBS Lab	1	2	3	4	5	6	7	Range	Spike value
T1C1 Ciclosporin A in µg/L								74 - 156	100
T2C2 Ciclosporin A in µg/L								445 - 903	600
T1C1 Tacrolimus in µg/L								4.4 -6.5	5
T2C2 Tacrolimus in µg/L								25.9 - 36.2	30
Patient Sample: Tacrolimus in µg/L								9.7 - 15.7	
T1C1+T2C2: Creatinin in µmol/L									77
Patient sample: Creatinin in µmol/L									118
Hematocrit T1C1 + T2C2									0.47
Hematocrit patient sample									0.34
Wholeblood									
T1C1 Ciclosporin A in µg/L								109 - 128	100
T2C2 Ciclosporin A in µg/L								689 - 777	600
T1C1 Tacrolimus in µg/L								5.0 - 5.6	5
T2C2 Tacrolimus in µg/L								30.7 - 34.5	30
Patient Sample: Tacrolimus in µg/L								11.0 - 12.5	

Table 9 – Percentage of reported values compared to the average – Round 2 – For DBS to average of whole blood

DBS Lab	1	2	3	4	5	6	7	Average (%)	Bias (%)	Presicion (rsd) (%)	Range (%)	Average (µg/L)
T1C1 CsA (%)								89.8	-10.2	30.4	64.3 - 135.4	115.2
T2C2 CsA (%)								89.5	-10.5	25.6	62.3 - 126.4	714.3
T1C1 Tac (%)								103.8	3.8	16.3	86.1 - 126.6	5.1
T2C2 Tac (%)								96.7	-3.3	11.7	80.7 - 112.7	32.1
Patient Tac (%)								110.6	10.6	15.8	85.5 - 138.9	11.3
Wholeblood												
T1C1 CsA (%)								100.0	0.0	5.6	94.4 - 111.1	115.2
T1C1 Tac (%)								100.0	0.0	5.3	96.9 - 109.6	5.1
T2C2 CsA (%)								100.0	0.0	4.4	96.6 - 108.8	714.3
T2C2 Tac (%)								100.0	0.0	3.9	95.5 - 107.3	32.1
Patient Tac (%)								100.0	0.0	4.9	97.3 - 110.7	11.3

	Within 15% of spiked value	
Yes	19	57.6%
No	14	42.4%
Total	33	100.0%

	Within 15% of spiked value	
Yes	35	100.0%
No	0	0.0%
Total	35	100.0%

	Within 26.5% of spiked value	
Yes	27	81.8%
No	6	18.2%
Total	33	100.0%

	Within 26.5% of spiked value	
Yes	100	100.0%
No	0	0.0%
Total	35	100.0%

Mogelijke verklaringen (1)

- Groot verschil in extractiemethoden

Table 7 Specifications of DBS extractions methods

	1	2	3	4	5	6	7
Is ZnSO4 used during extraction?	Yes	Yes	No	Yes	No	No	No
Is Ethanol used during extraction?	No	No	No	No	No	Yes	No
Is Methanol used during extraction? If yes, please provide ratio	Yes, MeOH:H2O 80:20	No	Yes, MeOH:H2O 100:50	Yes, MeOH: H2O 5:2	Yes, MeOH: Acetonitril 82:18	Yes,EtOH:MeOH 1:1	Yes, 80:20
Is acetonitrile used during extraction?	No	Yes (in IS)	No	No	Yes	No	No
Is sonication used during extraction?	No	No	Yes	Yes	No	Yes	Yes
If yes: sonication time in minutes			15	15		10	15
Is vortexing used during extraction?	Yes	Yes	No	Yes	No	Yes	Yes
If Yes: vortexing time in minutes	15	Seconds		10 sec		0.33	2
Do you use a roller during extraction? (Laboratory tube roller)	No	Yes	No	No	Yes	No	No
If yes: roller time in min		20			60		
Is centrifuging used during extraction?	Yes	Yes	No	No	No	No	No
If Yes: time and RPM?	13000 RPM 5 min	13000 RPM 2 min					
Do you have any other, relevant, specification to provide?						Eveporate and dissolve in mobile phase	Samples are put in -20 degrees celcius for 10 minutes post extraction

Mogelijke verklaringen (2)

- Groot verschil in extractiemethoden
- Verschil in punch grootte, type papier, kniptang/puncher, etc.
- Beperkte richtlijnen
- Klinische validatie studies
- DBS is ‘nieuw’

Gemaakte afspraken

- Punchen met Leidse puncher
- Sonificatie 15 minuten voor betere recovery
- Standaarden gemaakt uit vers bloed, spiken, 24 uur laten staan, spotten, 24 uur drogen

- In mei/juni ronde 3

Conclusie – We zijn er nog niet…

- Standaardisatie is nodig
- Ringonderzoek is nodig
- Samenwerking tussen centra blijft nodig

Met dank aan:

- DBS proficiency test research team:

Herman Veenhof, Remco Koster, Peter Zweipfenning, Daan Touw

- **Deelnemers KKGT ringonderzoek**