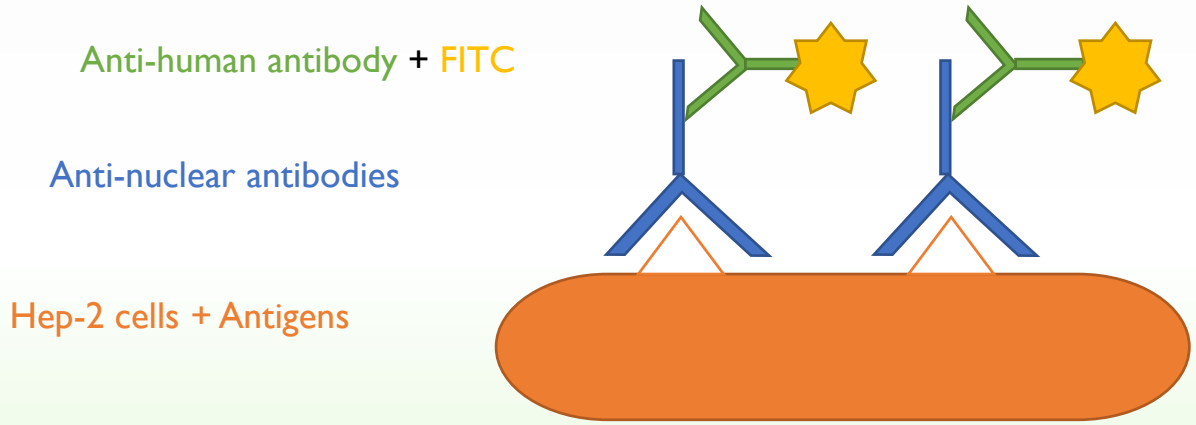
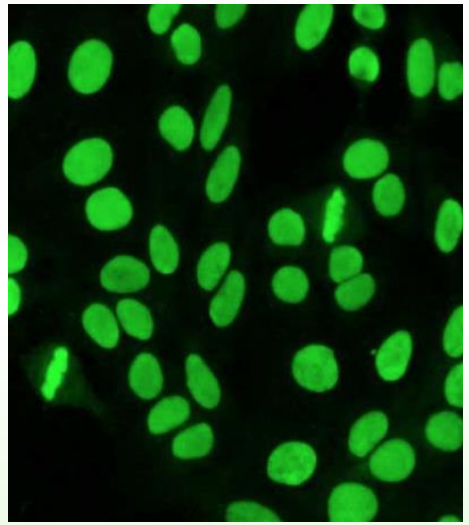
Optimizing an internal quality control program of automated ANA IIF

Laura Bogaert
Supervisor: Apr. Biol. S. Schouwers & Apr. Biol. L. Van Hoovels

Introduction

- ANA detection
  - Anti-nuclear antibodies
  - Diagnosis/screening of ANA associated rheumatic diseases
  - Indirect immunofluorescence assay (IIF)
    - HEp-2 cells





Introduction

- Disadvantages ANA IIF
  - High workload (time-consuming and laborious)
  - Need for microscopic expertise
  - Subjective visual reading
  - High intra- and inter-laboratory variance

- Automated microscopic analysis
  - More standardization
  - More harmonization?
  - **Quality assurance program for total ANA IIF process**

Introduction

- Quality assurance program for total ANA IIF process
  - Total ANA IIF process
    - Pre- to post-analytical phase
  - Objective iQC procedures
    - Automated ANA IIF
  - Quality indicators and iQC acceptance criteria

Questions

1. Defining usable quality indicators to reveal analytical and clinically significant errors in the total, automated ANA IIF process.
2. Evaluation of different quality indicators in an experimental setup.
3. Evaluation of different quality indicators in daily routine laboratory practice.

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Questions

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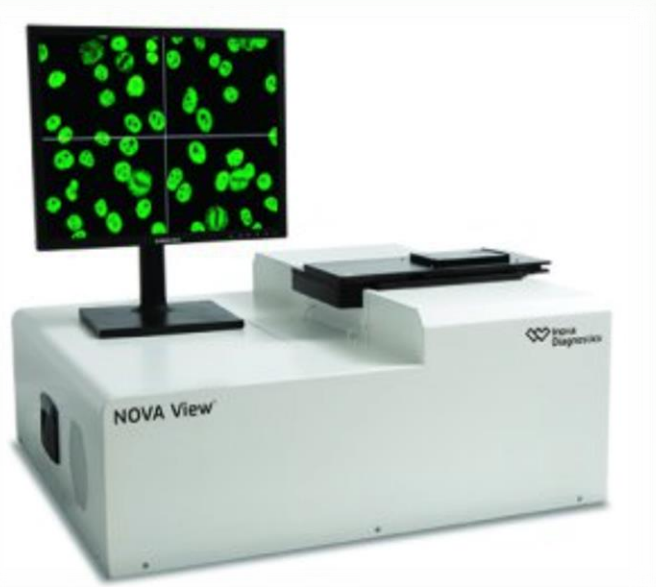
Defining usable quality indicators to reveal analytical and clinically significant errors in the total, automated ANA IIF process

- Materials and methods
  - QUANTA-Lyser – NOVA View system



Defining usable quality indicators to reveal analytical and clinically significant errors in the total, automated ANA IIF process

- Materials and methods
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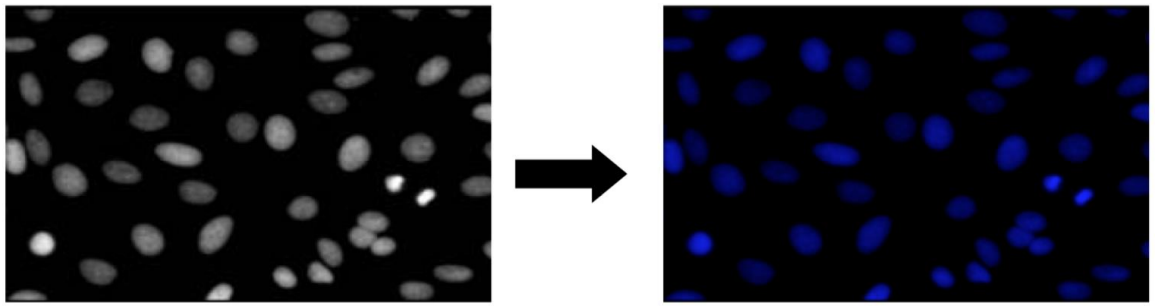

Figure 3-11A Focusing of cells, color conversion and image processing in the DAPI channel, selection of cells for analysis

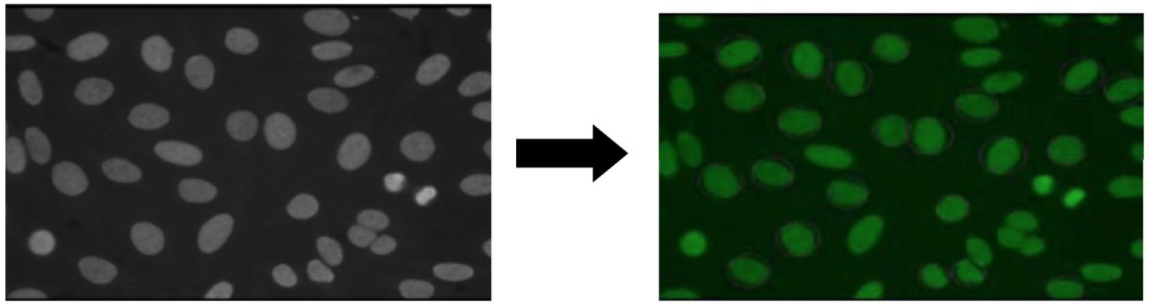

Figure 3-11B Fine focusing, switch to FITC channel, algorithm analysis and pattern recognition

INOVA Diagnostics, Inc. NOVA View – Automated iFA Microscope. Operators manual.

Defining usable quality indicators to reveal analytical and clinically significant errors in the total, automated ANA IIF process

- Materials and methods
  - Quality materials
    - Company iQC materials (NOVA Lite HEp-2 ANA kit)
      - Negative
      - Positive
    - Patient sample iQC materials
      - Negative
      - Positive (+/- LIU 200)
        - Speckled
        - Homogeneous

Defining usable quality indicators to reveal analytical and clinically significant errors in the total, automated ANA IIF process

- Materials and methods
  - Quality indicators

Quality indicators
LIU positive kit iQC
LIU negative kit iQC
LIU positive sample iQC (Speckled)
LIU positive sample iQC (Homogeneous)
LIU negative sample iQC
% positive ANA IIF patient samples/run
Median patient sample LIU/run
Mean patient sample LIU/run

Defining usable quality indicators to reveal analytical and clinically significant errors in the total, automated ANA IIF process

- Materials and methods
  - iQC acceptance criteria
    - Objective decision making
    - Based on the imprecision
      - Within-run reproducibility
      - Between-run reproducibility

Defining usable quality indicators to reveal analytical and clinically significant errors in the total, automated ANA IIF process

- Results
  - Total imprecision

CLSI EP5-A2 protocol (No outliers with Grubbs' test)					
	Negative kit iQC (LIU)	Positive kit iQC (LIU)	Negative sample iQC (LIU)	Positive sample iQC speckled (LIU)	Positive sample iQC homogeneous (LIU)
Mean	0	2133,4	13,9	189,4	264,8
Total imprecision (SD)	0	658,0	5,2	51,4	68,2
Total imprecision (CV%)	-	**30,8%**	**37,2%**	**27,1%**	**25,7%**

Defining usable quality indicators to reveal analytical and clinically significant errors in the total, automated ANA IIF process

- Results
  - Target values positive iQC materials

iQC target values									
%CV	Criteria	Positive kit iQC		Positive speckled sample iQC			Positive homogeneous sample iQC		
		SD (Intensity)	Range (Intensity)	SD (Intensity)	Range (Intensity)	SWT	SD (Intensity)	Range (Intensity)	SWT
25%	-	533,4	1600,1 – 2666,8	47,3	142,0 – 236,7	160 – 160	66,2	198,6 – 331,0	160 – 320
50%	Warning rule	1066,7	1066,7 – 3200,1	94,7	94,7 – 284,0	160 – 320	132,4	132,4 – 397,2	160 – 320
75%	Stop rule	1600,1	533,4 – 3733,5	142,0	47,3 – 331,4	0 – 320	198,6	66,2 – 463,4	80 – 320

1_{S2} (a single control measurement exceeds the mean +/- 2 CV% target) as a warning limit
1_{S3} (a single control measurement exceeds the mean +/- 3 CV% target) as stop limit

Defining usable quality indicators to reveal analytical and clinically significant errors in the total, automated ANA IIF process

- Results
  - Target values negative iQC materials

iQC target values				
%CV	Criteria	Negative sample iQC		
		SD (Intensity)	Range (Intensity)	SWT
25%	-	3,5	10,4 – 17,3	-
50%	Warning rule	6,9	6,9 – 20,8	-
75%	Stop rule	10,4	3,5 – 24,2	-.

I_{S2} (a single control measurement exceeds the mean +/- 2 CV% target) as a warning limit
I_{S3} (a single control measurement exceeds the mean +/- 3 CV% target) as stop limit

Defining usable quality indicators to reveal analytical and clinically significant errors in the total, automated ANA IIF process

- Results
  - Acceptance criteria for iQC

		Target value and coefficient of variation (CV)	Acceptance criteria
Process control	LIU positive kit iQC	• Pattern of initial ANA IIF analysis • Target LIU-value: >48 • Target CV: 25%	• Exact match of target pattern • Westgard rules (1_{S2} as a warning limit and 1_{S3} as stop limit)
	LIU negative kit iQC	• Negative on 1:80 dilution • Target LIU-value: ≤48 • Target CV: 25%	• Negative • Westgard rules (1_{S2} as a warning limit and 1_{S3} as stop limit)
	LIU positive sample iQC (speckled/homogeneous)	• Pattern of initial ANA IIF analysis • Target LIU-value: +/- 200 • Target CV: 25%	• Exact match of target pattern • Westgard rules (1_{S2} as a warning limit and 1_{S3} as stop limit)
	LIU negative sample iQC (patient pool)	• Negative on 1:80 dilution • Target LIU-value: ≤48 • Target CV: 25%	• Negative • Westgard rules (1_{S2} as a warning limit and 1_{S3} as stop limit)

Defining usable quality indicators to reveal analytical and clinically significant errors in the total, automated ANA IIF process

- Results
  - Acceptance criteria for iQC

		Target value and coefficient of variation (CV)	Acceptance criteria
Monitoring of patient results	% positive ANA IIF patient samples/run	• Target value: positive/negative ratio at 1:80 dilution of a real-life routine run • Target CV: 25%	• Westgard rules (1_{S2} as a warning limit and 1_{S3} as stop limit)
	Median patient sample LIU/run	• Target value: overall median of the 16 study patient samples (distribution of the LIU-values at 1:80 dilution of a real-life routine run) • Target CV: 25%	• Westgard rules (1_{S2} as a warning limit and 1_{S3} as stop limit)
	Mean patient sample LIU/run	• Target value: overall mean of the 16 study patient samples (distribution of the LIU-values at 1:80 dilution of a real-life routine run) • Target CV: 25%	• Westgard rules (1_{S2} as a warning limit and 1_{S3} as stop limit)

Questions

1. Defining usable quality indicators to reveal analytical and clinically significant errors in the total, automated ANA IIF process.
2. Evaluation of different quality indicators in an experimental setup.
3. Evaluation of different quality indicators in daily routine laboratory practice.

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Evaluation of different quality indicators in an experimental setup

- Materials and methods
  - Quality materials
  - 16 study samples
    - Simulation of real-life routine ANA IIF run
      - Positive/negative ratio
      - Distribution of LIU-values and SWT
    - Relative change of LIU (reference run)

Patient samples			
Study number	**LIU**	**ANA IIF Pattern**	**SWT**
Patient sample 1	36	-	-
Patient sample 2	24	-	-
Patient sample 3	32	-	-
Patient sample 4	25	-	-
Patient sample 5	33	-	-
Patient sample 6	10	-	-
Patient sample 7	9	-	-
Patient sample 8	55	Homogeneous	80
Patient sample 9	82	Speckled	80
Patient sample 10	120	Speckled	80
Patient sample 11	188	Speckled	160
Patient sample 12	213	Homogeneous	160
Patient sample 13	443	Homogeneous	320
Multicenter study sample 2	434	Speckled	320
Patient sample 14	1050	Homogeneous	640
Patient sample 15	2228	Speckled	1280

Evaluation of different quality indicators in an experimental setup

- Materials and methods
  - Artificial errors

Errors in the ANA IIF analytical process	
Pre-analytical problems	
1. Needle obstruction	• 5 µL sample volume in 790 µL PBS-buffer, instead of 10 µL + incubation of 18 µL sample dilution/kit iQC and 18 µL conjugate on slide instead of 35 µL
Analytical problems	
1. PBS-buffer dilution	• 1 bottle PBS-buffer in 2000 mL instead of 1 bottle in 1000 mL
2. Old PBS-buffer	• Use of PBS-buffer after 3 months. Insert claims 4 weeks stability after dilution
3. Old conjugate	• Use of conjugate 3 months after opening
4. Contrad dilution	• 8 mL Contrad in 1000 mL instead of 4 mL in 1000 mL
5. Sample wash step error	• 1 wash cycle with 1 mL instead of 3 wash cycles with 2 mL
6. Conjugate wash step error	• 1 wash cycle with 1 mL instead of 3 wash cycles with 2 mL
7. Needle contamination	• Absence of Contrad buffer (no rinsing liquid)
Post-analytical problems	
1. Final slide incubation >3h	• Slide more than 3 hours in PBS-buffer on QUANTA-Lyser before NOVA View analysis
2. Rescanning slide	• 5x rescanning of same slide

Evaluation of different quality indicators in an experimental setup

- Results

Effect of errors on iQC performance							
	Rescanning 5x				Slide incubation >3h PBS	Old conjugate 3m	Needle contamination
	Scan 2	Scan 3	Scan 4	Scan 5			
LIU Pos kit iQC	-11,1%	-13,3%	-19,7%	-26,3%	-9,4%	-1,8%	-0,8%
LIU Neg kit iQC	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%
LIU Pos sample iQC SP	-35,1%	-45,4%	-50,2%	-69,4%	-40,5%	-49,2%	194,7%
LIU Pos sample iQC HOM	-17,7%	-49,6%	-39,5%	-63,2%	-25,6%	-56,6%	134,9%
LIU Neg sample iQC	-16,7%	-11,1%	-33,3%	-47,2%	-11,1%	-44,4%	1276,0%
% positive ANA IIF Patient samples/run	-8,6%	-4,0%	-10,0%	-10,0%	0,0%	-10,0%	50,0%
Median patient sample LIU/run	-14,9%	-41,5%	-54,9%	-65,9%	-24,7%	-60,1%	166,6%
Mean patient sample LIU/run	-1,1%	-16,2%	-30,1%	-38,3%	-14,2%	-23,7%	46,2%
Δ titer step (≥ 2 steps)	0	0	0	0	0	0	2

Evaluation of different quality indicators in an experimental setup

- Results

Needle contamination					
	Intensity			Endpoint	
	Intensity reference	Intensity	Δ% (Scan - Scan(ref))	Endpoint reference	Endpoint
Pos kit iQC	2068	2052	-0,77%		
Neg kit iQC	0	1	/		
Neg sample iQC	10	15	50,00%		
Pos sample iQC SP	110	212	92,73%	160	160
Pos sample iQC HOM	163	105	-35,58%	160	160
Patient sample 1	32	25	-21,88%		
Patient sample 2	16	41	156,25%		
Patient sample 3	24	74	208,33%		80
Patient sample 4	19	55	189,47%		80
Patient sample 5	23	57	147,83%		80
Patient sample 6	11	20	81,82%		
Patient sample 7	8	28	250,00%		
Patient sample 8	52	100	92,31%	80	80
Patient sample 9	103	299	190,29%	160	320
Patient sample 10	98	307	213,27%	160	320
Patient sample 11	136	382	180,88%	160	320
Patient sample 12	291	608	108,93%	160	320
Patient sample 13	206	325	57,77%	160	320
Patient sample 14	837	976	16,61%	640	640
Patient sample 15	2272	2471	8,76%	1280	1280
Multicenter study sample 2	277	672	142,60%	160	320
Neg sample iQC (2)	7	228	3157,14%		160
Pos sample iQC SP (2)	99	404	308,08%	80	320
Pos sample iQC HOM (2)	131	587	348,09%	160	320

Needle contamination	
	Quality indicators
LIU Pos kit iQC	2052
LIU Neg kit iQC	1
LIU Pos sample iQC SP	308
LIU Pos sample iQC HOM	346
LIU Neg sample iQC	121,5
% positive ANA IIF Patient samples/run	75,00%
Median patient sample LIU/run	199,50
Mean patient sample LIU/run	402,50
Δ titer step (≥ 2 steps)	2
% Samples with Δ LIU (>50%)	75,00%
% Samples with Δ LIU (>75%)	66,67%

Evaluation of different quality indicators in an experimental setup

- Results

Effect of errors on iQC performance						
	Needle obstruction	Contrad dilution	Buffer dilution	Sample wash step	Conjugate wash step	Old buffer
LIU Pos kit iQC	1,4%	6,2%	-2,4%	-10,5%	-2,5%	-4,2%
LIU Neg kit iQC	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%
LIU Pos sample iQC SP	-85,7%	-30,4%	105,9%	-43,8%	43,1%	-8,1%
LIU Pos sample iQC HOM	-92,2%	-33,1%	123,6%	-39,2%	64,6%	-12,8%
LIU Neg sample iQC	-100,0%	-25,0%	1430,6%	-22,2%	1,9%	1,9%
% positive ANA IIF Patient samples/run	-62,5%	0,0%	60,0%	10,0%	25,0%	0,0%
Median patient sample LIU/run	-86,0%	-33,2%	303,4%	-37,5%	89,1%	-9,1%
Mean patient sample LIU/run	-43,3%	-13,8%	92,6%	-8,3%	22,7%	3,4%
Δ titer step (≥ 2 steps)	9	0	9	0	0	0

Questions

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2. Evaluation of different quality indicators in an experimental setup.
3. Evaluation of different quality indicators in daily routine laboratory practice.

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Evaluation of different quality indicators in daily routine laboratory practice

- Materials and methods
  - iQC acceptance criteria
    - Imprecision 10 consecutive, stable routine ANA IIF runs
  - Quality indicators
    - 3 predefined periods of routine ANA IIF analysis (OLV Hospital Aalst in 2017)
      - Stable period (2/08/2017-11/09/2017)
      - Two periods containing a methodological or technical intervention (17/05/2017-28/06/2017 and 15/11/2017-31/12/2017)
    - Predefined acceptance criteria (CV 25%)
      - 1_{S2} (a single control measurement exceeds the mean +/- 2 CV% target) as a warning limit
      - 1_{S3} (a single control measurement exceeds the mean +/- 3 CV% target) as stop limit

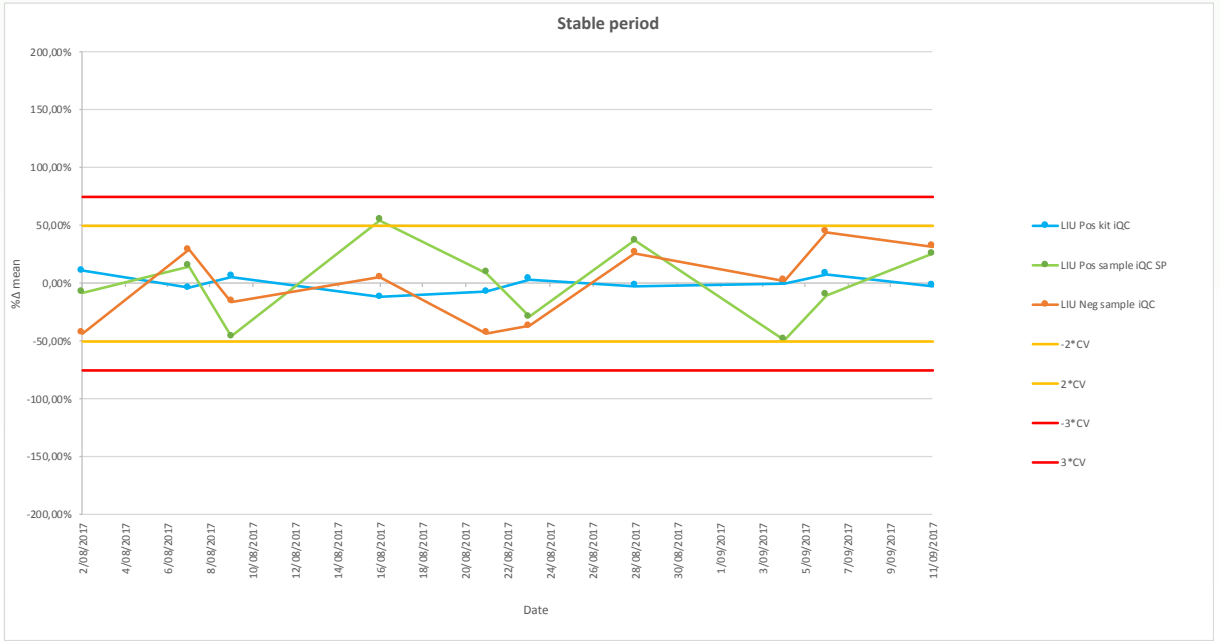
Evaluation of different quality indicators in daily routine laboratory practice

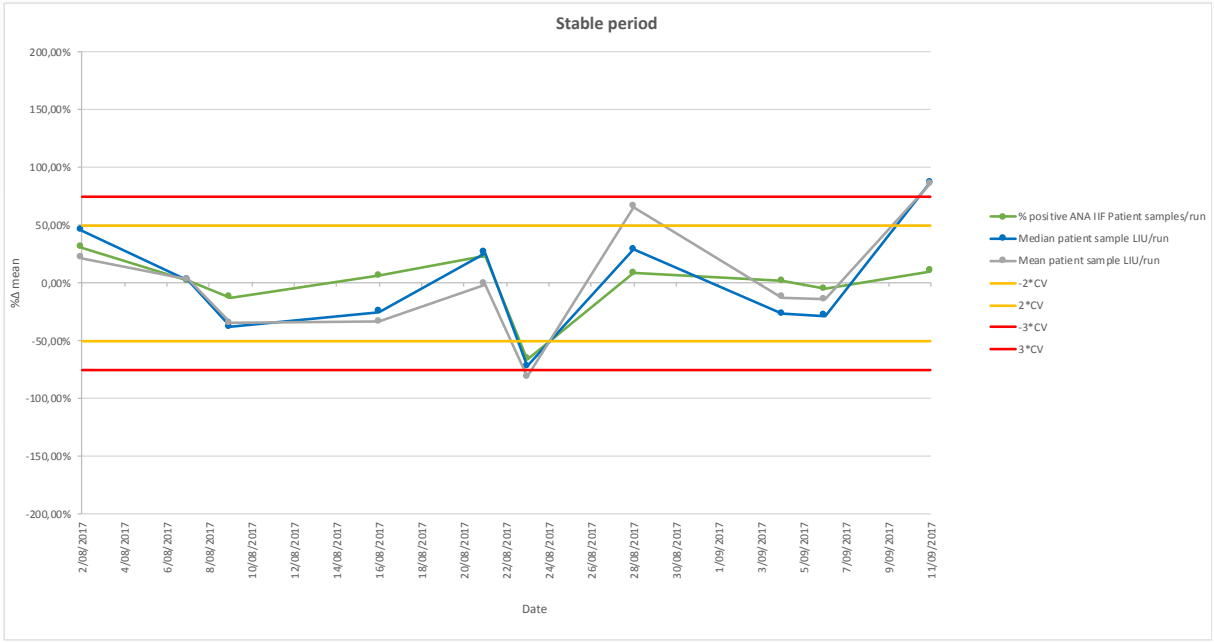
- Results
  - iQC acceptance criteria

Imprecision quality indicators (10 stable routine runs)							
	LIU Positive kit iQC	LIU Negative kit iQC	LIU Positive sample iQC speckled	LIU Negative sample iQC	% positive ANA IIF patient samples/run	Median patient sample LIU/run	Mean patient sample LIU/run
Mean	2089,2	0,2	269,6	33,3	0,6	79,1	237,6
SD	148,0	0,4	93,5	11,0	0,2	37,3	115,8
CV (%)	**7,1%**	**210,8%**	**34,7%**	**33,1%**	**26,4%**	**47,1%**	**48,7%**

Evaluation of different quality indicators in daily routine laboratory practice

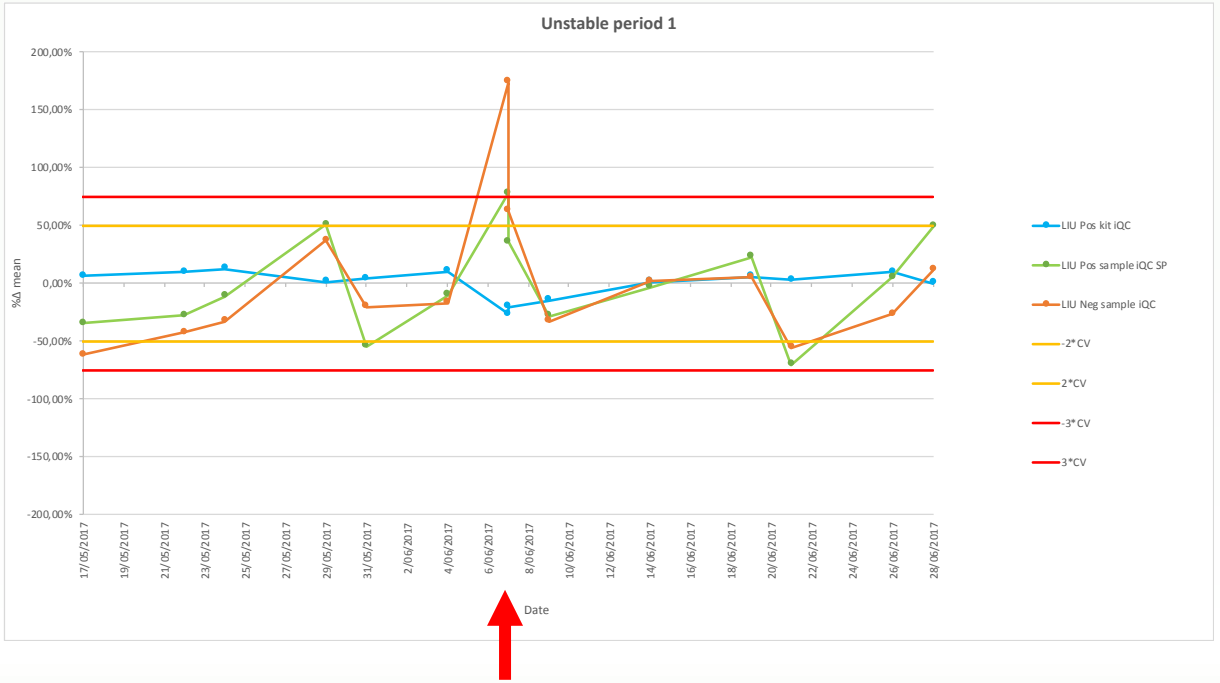
- Results
  - Quality indicators

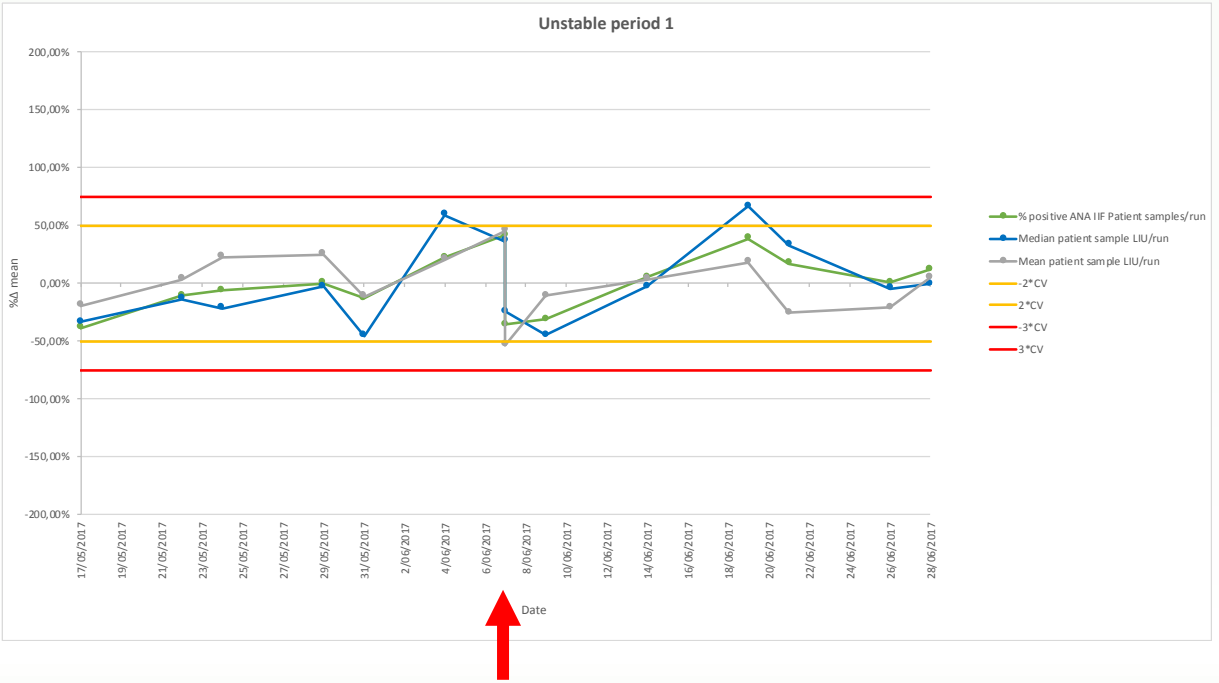




Evaluation of different quality indicators in daily routine laboratory practice

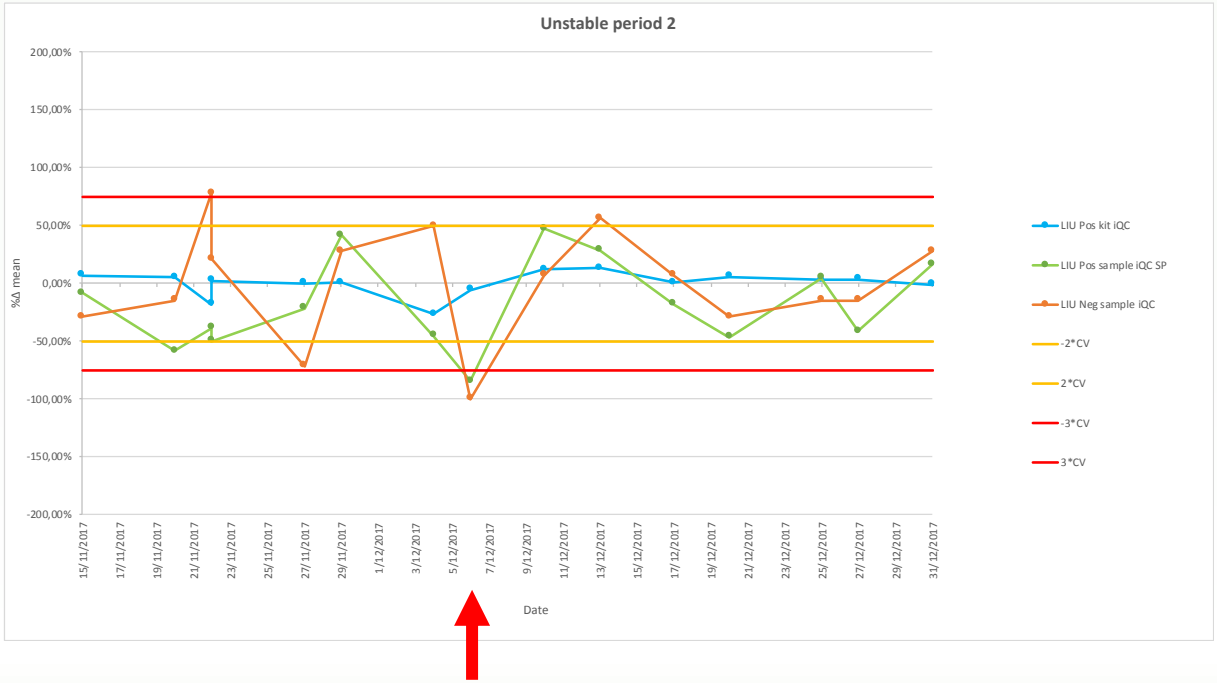
- Results
  - Quality indicators

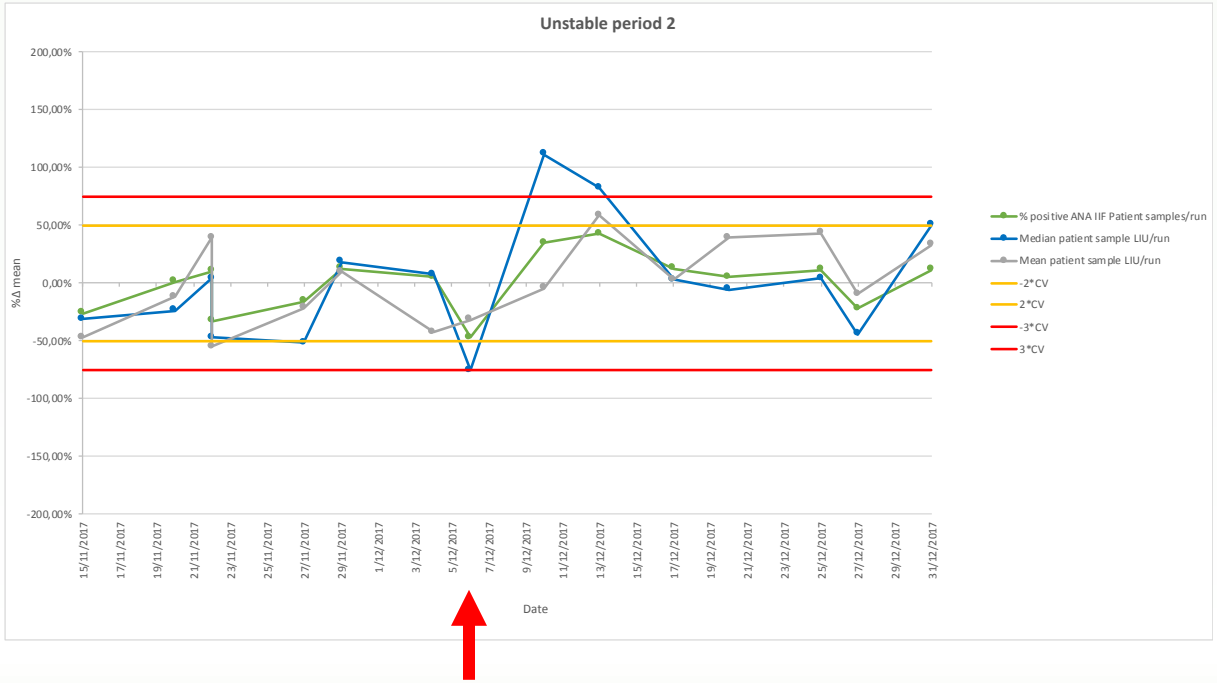




Evaluation of different quality indicators in daily routine laboratory practice

- Results
  - Quality indicators





Discussion

- ANA IIF analysis
  - Intra- and inter-laboratory variation
  - Automated microscopic analysis
    - Harmonization?

- iQC program
  - Kit iQC materials are of little significance as quality indicators
  - Additional quality indicators are necessary
    1. Assurance of whole ANA IIF process (dilution-result interpretation)
    2. Patient-derived iQC material with a moderate FI (1:160 titer)

Discussion

- iQC program
  - Process control = follow-up of LIU values + Westgard multi-rules
    - I_{S2} as **warning** limit = 2*CV% (50%) (iQC trend analysis)
    - I_{S3} as **stop** limit = 3*CV% (75%) (root cause analysis and review of acceptance of the run)
  - Monitoring of patient results
    - % ANA positive samples per run
    - Median LIU per run
      - High variability (demographic variations)
  - Decisions can never be taken by interpreting only one quality indicator

Discussion

- Automated ANA IIF analysis = harmonization?
  - High inter-assay variability inherent to ANA IFF analysis despite automation (QUANTA Lyser and NOVA View)
  - Need for more efforts in harmonizing automated ANA IIF analysis

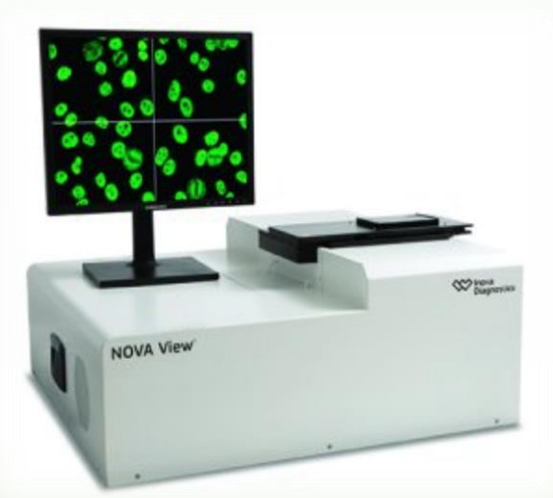
Conclusion

1. Analytical and clinical process control by monitoring the LIU values of the **company and patient-derived negative and positive iQC control materials**. Patient-derived iQC samples are necessary to ensure that the whole ANA IIF analysis process is controlled, from dilution up to result interpretation.
2. Well-chosen **target value of iQC control materials** to detect clinically important iQC violations.
3. A **target CV for the iQC of 25% can be used, with I_{S3} as stop limit**. The I_{S2} can be used as warning limit, only to encourage trend analysis or to indicate that further follow-up is required without a clinical problem at that moment.
4. iQC monitoring of patient results based on the **percentage ANA positive samples per run and on the median-LIU per run**. One run must contain at least 20 patient screening (1:80 dilution) samples in order to calculate these quality indicators.
5. In daily routine practice, **decisions can never be taken by interpreting only one quality indicator**.

To do

1. Discussion of the proposed quality procedure during a user meeting with the other NOVA View users.
2. Further refinement of the quality procedure in daily practice.
3. Supporting national and international initiatives regarding ANA IIF harmonization.

Questions?



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