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EVALUATION OF THE COBAS LIAT® (ROCHE®) AND THE LIAISON MDX® (DIASORIN®)

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Intern in Clinical Pathology
Corata, 20-21 September 2018

Presentation Plan

- Evaluation of the cobas®Liat® Influenza A, B & RSV assay (Roche®) and the Simplexa® Flu A/B & RSV direct kit (Diasorin®)
- Evaluation of the cobas® cdiff (Roche®) and the Simplexa® C. difficile Universal direct kit (Diasorin®) for the detection of toxigenic *Clostridium difficile* in routine stool samples
- cobas® Liat® - Liaison MDX®: Pros and Cons

1. Evaluation of the cobas®Liat® Influenza A, B & RSV assay (Roche®) and the Simplexa® Flu A/B & RSV direct kit (Diasorin®)

Online : Labmanager (26/10/2017)

Online : Roche (26/10/2017)

1.Introduction : screening strategy for detection of influenza A/B & RSV at the lab

- Influenza A/B :
  - Alere®i Influenza A & B
  - Sensitivity[a] :
    - Influenza A : 59.1 % ➔ 97.8 %
    - Influenza B : 62.5 % ➔ 100 %
  - Specificity[a] :
    - Influenza A : 53.3 % ➔ 100 %
    - Influenza B : 53.6 % ➔ 100 %
  - Results available in 13 minutes

Online: Alere (30/10/17)

Technology :

isothermal nicking enzyme amplification reaction (molecular beacon)

Target :

Influenza A : a region of the polymerase basic protein 2 gene
Influenza B : a region of the polymerase acid protein gene

- RSV :
  - TRU RSV® (Meridian)
  - Sensitivity [b] : 71 .7 % ➔ 92.3 %
  - Specificity [b] : 81.1 % ➔ 96.2 %
  - Results available in 20 minutes
  - Only for patients below 2 years of age!

Online: Meridian (30/10/17)

Technology :

Rapid qualitative immunoassay

[a]CADTH 2016
[b]According to manufacturer

2. Material and Method : study design

- Multi-center retrospective study conducted from July to August 2017 at the « Hôpital Civil Marie Curie » in Charleroi and « Clinique Saint-Luc » in Brussels

- Inclusion criteria
  - Patients with Influenza-like symptoms in the previous 48-hour period during the 2015-2016 and 2014-2015 respiratory flu seasons (samples frozen at -80°C)
  - Performed in the case of a specific request from the physician

- Exclusion criteria
  - Insufficient material (< 1mL)

- Type of samples (Bias?)
  - Hôpital Civil Marie Curie (n = 52)
    - nasopharyngeal aspirate/swabs
      collected using Copan eswab®(!kids!)
  - Clinique Saint-Luc (n = 37)
    - Nasal and nasopharyngeal aspirate/swabs resuspended in PBS or UTM
    - Bronchoalveolar lavage and entotracheal aspirate resuspended in PBS or UTM

Online : Copan eswab®, Copan® (30/10/17)

2. Material and Method : study design

Online : Labmanager (26/10/2017)

Online : Roche (26/10/2017)

Online: Alere (30/10/17)

Discordant results ➔ Cepheid Xpert Flu/RSV XC

2. Material and Method : Timing!

- Alere i®:
  - Multiple steps and components
  - Waiting time of 3 minutes
  - Results available in 13 minutes
  - Random Access

Online: Alere (30/10/17)

- Roche Liat®:
  - Run configuration : 1 minute
  - Analysis: 20 minutes
  - Random Access

Online : Roche (27/10/17)

- Liaison MDX®:
  - Run configuration : 1 minute/sample (batch!)
  - Analysis : 60-70 minutes (8 samples MAX)

Online : Diasorin (27/10/17)

3. Results : impact of media / sample type

- Impact of viscosity (Hôpital Civil Marie Curie) ?
  - Cobas Liat ® / Liaison MDX® - invalid :
    - 5 of 13 / 0 of 13 nasopharyngeal aspirate without transport media (38.4% / 0%) and various viscosity
    - 2 of 39 / 1 of 39 nasopharyngeal aspirate/swabs collected using Copan eswab® (5.1% / 2.5 %)
  - Hypothesis :
    - Cobas Liat ®: because of the viscosity, the plungers and clamps cannot proprely compress the Liat tube segments to move the sample from one segment to another and/or control reaction conditions
    - Liaison MDX® : high speed centrifugation moves all sample to reaction chamber

Online : Roche (30/10/2017)

3. Results : impact of media / sample type

- Impact of transport media (Clinique Saint-Luc) :
  - Cobas Liat ® / Liaison MDX® - invalid :
    - 0 of 19 / 7 of 19 Nasal and nasopharyngeal aspirate/swabs resuspended in PBS (0% / 37 %)

      ➔ All samples were resuspended in UTM for the rest of the study

    - 2 of 37 / 0 of 37 Nasal and nasopharyngeal aspirate/swabs resuspended in UTM (5.4 % / 0 %)
  - Hypothesis :
    - Cobas Liat ® more robust to transport media nature and/or salt concentration when compared to Liaison MDX®?

3. Results : Influenza A

- Clinical Performance :
  - N = 89
  - Disease prevalence = 37.5 %

	invalid	sensitivity	Specificity
Cobas Liat® (%)	5 + 4	100 (89.7 – 100)	89.80 (77.8 – 96.6)
Alere i® (%)	…	100 (89.7 – 100)	93.33 (81.7 – 98.6)
Liaison MDX® (%)	7 + 1	100 (89.4 – 100)	100 (93,4 – 100)

- Invalid
  - Cobas Liat® : viscosity ! ≈ 4.4 %
  - Liaison MDX®: Composition of transport media ! ≈ 1.1 %
  - Alere i®: retrospective results…

- Discordant results

(Reference : Cepheid® Flu A/B & RSV)

  - Cobas Liat® : 5 false positive
  - Liaison MDX®: Perfect
  - Alere i®: 3 false positive

3. Results : Influenza B

- Clinical Performance :
  - N = 89
  - Disease prevalence = 14.6 %

	invalid	sensitivity	Specificity
Cobas Liat®$(%)	5 + 4	100 (78.2 – 100)	100 (94.3 – 100)
Alere i® (%)	…	100 (78.2 – 100)	90 (80 – 96.3)
Liaison MDX® (%)	7 + 1	86.7 (60 – 98.3)	95.5 (87.8 – 99.1)

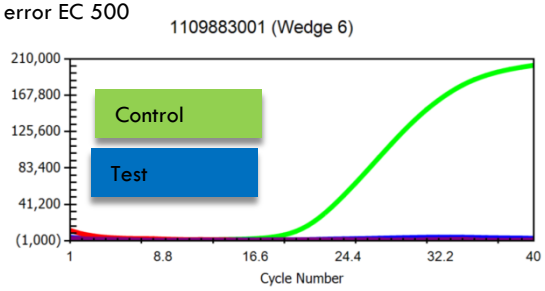
- Discordant results

(Reference : Cepheid® Flu A/B & RSV)

- Cobas Liat® : perfect
- Liaison MDX®:
  - 2 false negative
  - 3 false positive

1. First run ➔ POS
Rerun ➔ NEG

2 and 3: error EC 500



- Alere i®: 6 false positive

3. Results : RSV

- Clinical Performance :
  - N = 89
  - Disease prevalence = 10.1 %

	invalid	sensitivity	Specificity
Cobas (%)	5 + 4	100 (66.4 – 100)	87 (76.7 – 93.9)
Liaison MDX® (%)	7 + 1	77.8 (40 – 97.2)	100 (94 – 100)

- Discordant results

  (Reference : Cepheid® Flu A/B & RSV)

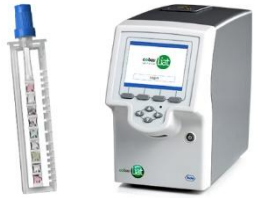
  – Cobas Liat® : 9 false positive

  – Liaison MDX®: 2 false negative

- Cross-reaction for Cobas Liat® ?

  – « false positive » positive for adenovirus (n = 2) MPV (n = 1) and parainfluenza (n = 1)

4. Discussion : Comparison of clinical performance with other published data - Cobas Liat®



1 **Multi-center evaluation of the cobas® Liat® Influenza A/B & RSV Assay for rapid point of care diagnosis** N = 1361

Jane Gibson[1], Elissa M Schechter-Perkins[2], Patricia Mitchell[2], Sharon Mace[4] Yu Tian[3], Kemi Williams[3], Robert Luo[3], Belinda Yen-Lieberman[4]

2 **Direct Comparison of Alere™ i and cobas® Liat Influenza A and B Tests for Rapid Detection of Influenza Virus Infection** N = 129

Frederick S. Nolte[a#], Lori Gauld[b], and Susan B. Barrett[b]

3 **Direct Detection of Influenza A and B Viruses in Less Than 20 Minutes Using a Commercially Available Rapid PCR Assay** N = 197

Matthew J. Binnicker, Mark J. Espy, Cole L. Irish, Emily A. Vetter

4 **LAB-IN-A-TUBE: REAL-TIME MOLECULAR POINT-OF-CARE DIAGNOSTICS FOR INFLUENZA A AND B USING THE COBAS® LIAT® SYSTEM†** N = 121

Willem JG Melchers[1*], Judith Kuijpers[1], Joanna Sickler[2], Janette Rahamat-Langendoen[1]

5 Original article

European Journal of Microbiology and Immunology 5 (2015) 4, pp. 236–24
DOI: 10.1556/1886.2015.0004

PERFORMANCE OF THE COBAS® INFLUENZA A/B ASSAY FOR RAPID PCR-BASED DETECTION OF INFLUENZA COMPARED TO PRODESSE ProFlu+ AND VIRAL CULTURE N = 123

L. Chen[1], Y. Tian[1], S. Chen[1], O. Liesenfeld[2,*]

6 **Diagnostic accuracy of the real-time PCR cobas® Liat® Influenza A/B assay and the Alere i Influenza A&B NEAR isothermal nucleic acid amplification assay for the detection of influenza using adult nasopharyngeal specimens** N = 87

Stephen Young[a,*], Patrick Illescas[a], Joclin Nicasio[a], Joanna Sickler[b]

7 Comparison of Six Sample-to-Answer Influenza A/B and RSV Nucleic Acid Amplification Assays Using Respiratory Specimens from Children N = 225

- Influenza A :
  - Sensitivity : 97.7 ➔ 100 % (100 %)
  - Specificity : 97.5 ➔ 100 % (89.8 %)
- Influenza B :
  - Sensitivity : 94.4 ➔ 100 % (100 %)
  - Specificity : 99.4 ➔ 100 % (100 %)
- RSV :
  - Sensitivity : 96.8 ➔ 98.1 %(100 %)
  - Specificity : 98.8 ➔ 99.4 % (87 %)

4. Discussion : Comparison of clinical performance with other published data – Liaison MDX®

1

Comparison of Six Sample-to-Answer Influenza A/B and RSV Nucleic Acid Amplification Assays Using Respiratory Specimens from Children

N = 225

2

Evaluation of Simplexa Flu A/B & RSV for Direct Detection of Influenza Viruses (A and B) and Respiratory Syncytial Virus in Patient Clinical Samples

Musa Hindiyeh,[a] Liat Kolet,[a] Tal Meningher,[a,b] Merav Weil,[c] Ella Mendelson,[a,d] Michal Mandelboim[a]

N = 170

3

Performance of the Simplexa™ Flu A/B & RSV Direct Kit on respiratory samples collected in saline solution

Malin J. Svensson, Ingrid Lind, Benita Zweygberg Wirgart, Maria Rotzén ÖStlund & Jan Albert

N = 210

4

Evaluation of the Simplexa Flu A/B and RSV Test for the Rapid Detection of Influenza Viruses

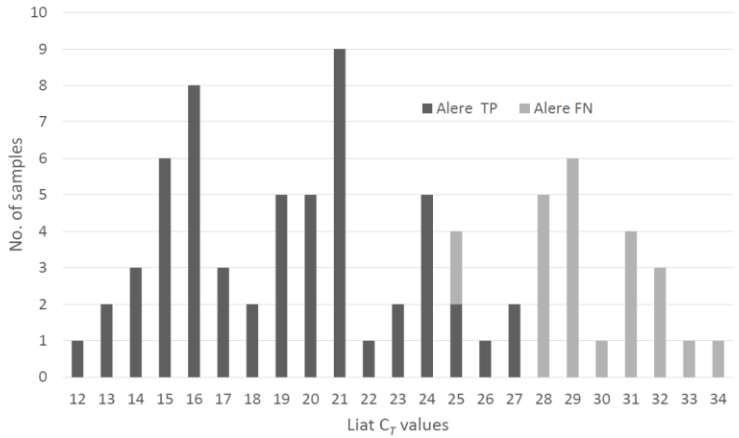
Sun-Young Ko,[1] Jin Woo Jang,[1] Dae Jin Song,[2] Chae Seung Lim,[1*] and Woo Joo Kim[3**]

N = 241

- Influenza A :
  - Sensitivity : 92.2 ➔ 100 % (100 %)
  - Specificity : 100 % (100 %)
- Influenza B :
  - Sensitivity : 85.4 ➔ 100 % (86.7 %)
  - Specificity 99.4 ➔ 100 % (95.5 %)
- RSV :
  - Sensitivity : 87 ➔ 95.1 % (77.8 %)
  - Specificity : 99.4 ➔ 99.6 % (100 %)

4. Discussion : Cobas Liat® *vs* Liaison MDX® and Alere i® (1)

- Alere i® :



Nolte et al. (2016)

- Sensitivities and specificities varying from approximately 55 to 100 %
- Impact of dilution ?
  ➔ 200µL vs 200µL in 2.5 mL
- Bias in the cohort? (Alere i®: 90 ➔ 100%)

- Liaison MDX®:

TABLE 1 Comparison of the Roche Cobas Liat and Focus Simplexa Direct for detection of influenza A

	Focus Simplexa influenza A			
Cobas Liat influenza A	No. positive	No. negative	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)
Positive	123	0	99.2 (95.1–99.9)	100 (94.0–100)
Negative	1	73		

TABLE 2 Comparison of the Roche Cobas Liat and Focus Simplexa Direct for detection of influenza B

	Focus Simplexa influenza B			
Cobas Liat influenza B	No. positive	No. negative	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)
Positive	23	0	100 (83.1–100)	100 (97.4–100)
Negative	0	174		

Binnicker et al. (2015)

4. Discussion : Cobas Liat® *vs* Liaison MDX® and Alere i® (1)

- Liaison MDX®:

		Fusion	Aries	Liat	Xpert	Simplexa	Biofire RP
FluA	TP	75	74	75	74	74	75
	TN	150	150	150	149	150	150
	FP	0	0	0	1	0	0
	FN	0	1	0	1	1	0
	Sensitivity (95% CI)	100% (93.9 – 100)	98.6% (91.7 – 99.9)	100.0% (93.9 – 100)	98.6% (91.7 – 99.9)	98.63% (91.7 – 99.9)	100% (93.9 – 100)
	Specificity (95% CI)	100% (96.8 – 100)	100% (96.8 – 100)	100% (96.8 – 100)	99.3% (95.7 –99.9)	100% (96.8 – 100)	100% (96.8 – 100)
	FN Subtype	NA	pH1N1	NA	H3N2	H3N2	NA
FluB	TP	48	45	47	47	41	46
	TN	177	176	174	176	176	173
	FP	0	1	3	1	1	4
	FN	0	3	1	1	7	2
	Sensitivity (95% CI)	100% (90.7 – 100)	93.7% (81.8 – 98.3)	97.9% (87.5 – 99.8)	97.9% (87.5 – 99.8)	85.4% (71.6 – 93.5)	95.8% (84.6 – 99.2)
	Specificity (95% CI)	100% (97.3 – 100)	99.4% (96.4 – 99.9)	98.3% (94.7 – 99.6)	99.4% (96.3 – 99.9)	99.4% (96.4 – 99.9)	97.7% (93.9 – 99.2)
	FN Subtype	None	Yamagata (3)	Yamagata	Yamagata	Yamagata (5) Victoria (2)	Yamagata (2)
RSV	TP	52	51	53	53	47	51
	TN	171	171	170	170	170	171
	FP	0	0	1	1	1	0
	FN	2	3	1	1	7	3
	Sensitivity (95% CI)	96.3% (86.2 – 99.3)	94.4% (83.6 – 98.5)	98.1% (88.8 – 99.9)	98.1% (88.8 – 99.9)	87% (74.5 – 94.2)	94.4% (83.6 – 98.5)
	Specificity (95% CI)	100% (97.2 – 100)	100% (97.2 – 100)	99.4% (96.3 – 99.9)	99.4% (96.3 – 99.9)	99.4% (96.3 – 99.9)	100% (97.2 – 100)
	FN Subtype	Not performed	Not performe	Not performed	Not performed	Not performed	Not performed

#TP- true positive, TN- true negative, FP - false positive, FN - false negative

Barnerjee et al. (2018)

4. Discussion : Turn Around Time (TAT)

- On an average, 10 to 20% of the world's population is affected by seasonal epidemic influenza each year, resulting in 3 to 5 million cases of severe illness and up to 500,000 deaths
- Common signs and symptoms of influenza (fever, dry cough, headache, nasal congestion, …) overlap with those of other bacterial and viral infections, making accurate diagnosis and provision of appropriate treatment difficult based on symptoms alone
- The greatest clinical benefit of antiviral treatments (e.g. neuraminidase inhibitors) higher when initiated within 48 hours of the onset of infection
- Major benefits of reduced TATs for viral respiratory infections are appropriate and effective use of anti-viral therapies, public health notification and tracking and prevention of unnecessary use of antibiotics, hospital procedures and laboratory tests
- TAT cobas Liat® Influenza A/B & RSV assay: 20 minutes (total hands-on time to start the test is ~1 min) + "random Access"

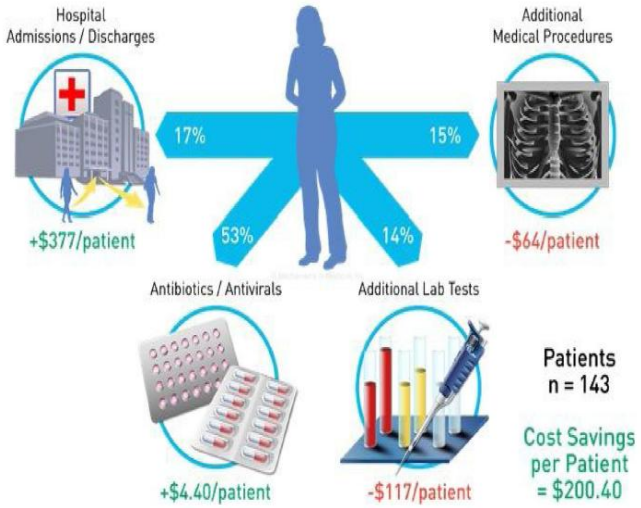
  >< TAT Liaison MDX®: 72 minutes (1 min per sample) + Batch (8 samples)

4. Discussion : Changes in patient management ?

Clinical decision making in the emergency department setting using rapid PCR: Results of the CLADE study group

Glen T. Hansen[a,b,c,*], Johanna Moore[d], Emily Herding[e], Tami Gooch[a], Diane Hirigoyen[a], Kevan Hanson[a], Marcia Deike[a]

Journal of Clinical Virology 102 (2018) 42–49



- Inclusion criteria : respiratory disease (only!)
  - CDC ILI definition
  - N = 143
- Pre and post influenza diagnosis survey
  - Disease prevalence = 43% (mainly Influenza B)
  - Physician sensitivity/specificity = 36% / 85%
  - Changes in patient management = 61% (87/143)
  - > 30 minutes! (292...!)

Table 3
Impact of Rapid Influenza testing on clinical decision making in the ED for suspected Influenza Patients (n = 143).

Clinical Touchpoint	Total cases impacted (%) 61%* (87/143)	% reduction in utilization/change in discharge	% increase in utilization or admission
Antimicrobial[!] prescribing total	58% (83/143)	10% (14/143) ↓	15% (21/143) ↑
Antibiotic prescribing	33.5% (48/143)	9% (13/143) ↓	33.6% (48/143) ↑
Antiviral prescribing	39.2% (56/143)	24.5% (35/143) ↓	14.7% (21/143) ↑
Medical Procedures/Imaging	15.4% (22/143)	2.1% (3/143) ↓	13.2% (19/143) ↑
Laboratory studies	14% (20/143)	2.8% (4/143) ↓	11.1% (16/143) ↑
Hospital Admission/Discharge	18% (26/143)	10.5% (15/143) ↓	7.7% (11/143) ↑

4. Discussion : Reduction of unnecessery antibiotics?

Routine molecular point-of-care testing for respiratory viruses in adults presenting to hospital with acute respiratory illness (ResPOC): a pragmatic, open-label, randomised controlled trial

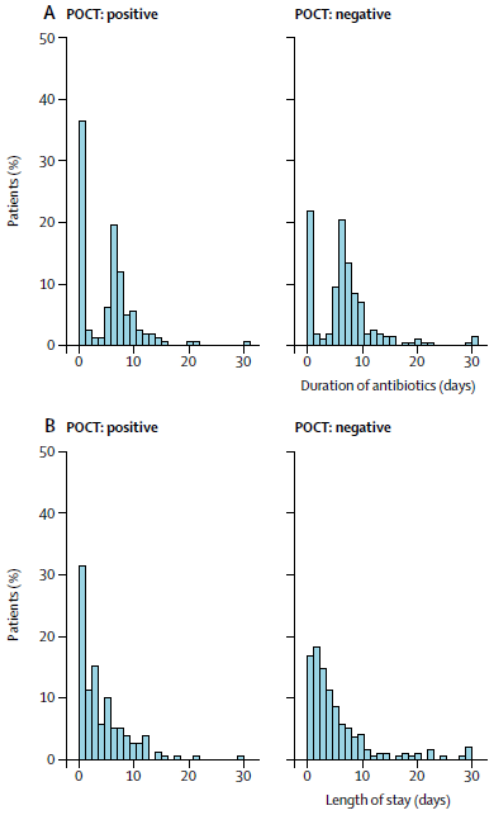
Nathan J Brendish, Ahalya K Malachira, Lawrence Armstrong, Rebecca Houghton, Sandra Aitken, Esther Nyimbili, Sean Ewings, Patrick J Lillie, Tristan W Clark

Lancet Respir Med 2017; 5: 401–11

	POCT (n=360)	Control (n=354)	Risk difference (95% CI)	Unadjusted odds ratio (95% CI)	Adjusted odds ratio (95% CI)	Number needed to test (95% CI)	p value
All antibiotics							
Antibiotics given	301 (84%)	294 (83%)	0·6% (−4·9 to 6·0)	1·04 (0·70 to 1·54)	0·99 (0·57 to 1·70)	..	0·96*
Single dose only	31/301 (10%)	10/294 (3%)	6·9% (2·9 to 11·0)	3·26 (1·59 to 6·68)	..	15 (9 to 35)†	0·0010
Given for <48 h	50/301 (17%)	26/294 (9%)	7·8% (2·5 to 13·1)	2·05 (1·40 to 3·39)	..	13 (8 to 41)‡	0·0047
Duration (days)	7·2 (5·1)	7·7 (4·9)	−0·4 (−1·2 to 0·4)§	0·95 (0·85 to 1·05)¶	0·91 (0·80 to 1·04)‖	..	0·17*

- Inclusion criteria :
  - N = 714
  - acute respiratory illness and/or fever >37.5
  - illness duration < 7 days
- Results :
  - Impact on antibiotic use
  - Asthma and IECOPD
  - Length of hospitalization and duration of antibiotics in the POCT subgroup
  - Rate of adverse outcome

Asthma or IECOPD	143 (40%)	140 (40%)	..	..	..
Antibiotics given	118/143 (83%)	111/140 (79%)	3·2% (−6·0 to 12·4)	1·23 (0·68 to 2·24)	0·55
Single dose only	21/118 (18%)	6/111 (5%)	12·4% (4·1 to 20·8)	3·79 (1·47 to 9·78)	0·0041
Given for <48 h	29/118 (25%)	7/111 (6%)	18·3% (9·0 to 27·4)	4·84 (2·02 to 11·59)	0·0002
Duration of antibiotics (days)	5·3 (3·4)	7·1 (4·5)	−1·8 (−2·8 to −0·8)	..	0·0008
Length of hospital stay (days)	4·0 (3·5)	5·4 (5·5)	−1·4 (−2·5 to −0·2)	..	0·0186



4. Discussion : Study drawback (1)

- Gibson et al. (2017) : Prodesse Proflu+ (Hologic®)

cobas® Liat® Ct Range	Reference Test % Positive		
	Influenza A	Influenza B	RSV
< 25	100% (201/201)	100% (116/116)	99% (155/156)
25 - 30	86% (57/66)	100% (20/20)	91% (21/23)
> 30	26% (9/34)	67% (8/12)	35% (8/23)

Cobas Liat results confirmed in **49 %** of cases by sequencing

- Chen et al. (2015) :

cobas Liat® Ct	Prodesse Proflu+ (Hologic®)	
	Influenza A	Influenza B
<25	100 % (n =108/108)	100 % (n = 108/108)
25 - 30	94 % (n = 45/48)	94 % (n= 33/35)
> 30	80 % (n = 21/26)	20 % (n = 1/5)

- Cobas Liat results confirmed in **45 %** of cases by sequencing
- On average, Cobas Liat® Ct values were lower than those in the Pro- Flu+ assay by 3.2 for influenza A, and 3.7 for influenza B (better sensitivity? Better efficiency?)

- Roth et al. (2015) : Comparison of results to a "home-made" PCR
  - Cobas Liat® Ct < 36 = agreement of 100% and 95 % for influenza A and B respecticely
  - Cobas Liat® Ct > 36 = agreement decreases to 50 % (4/8) and 83 % (5/6) for influ A and B

4. Discussion : Study drawback (2)

Dilution		Genexpert (influenza)		liat (influenza)
			Ct	
pure	influenza A	detected	23/25	detected
10		non testé		detected
1,00E+02		non testé		detected
1,00E+03		detected	34,8/37,5	detected
1,00E+04		ND		detected
2,00E+04		ND		detected
4,00E+04		ND		detected
8,00E+04		ND		ND
1,00E+05		ND		ND

Dilution		Genexpert (influenza)		liat (influenza)
			Ct	
pure	influenza A	detected	24/25	detected
10		non testé		detected
1,00E+02		non testé		detected
1,00E+03		detected	34,4/39	detected
1,00E+04		ND		detected
2,00E+04		ND		detected
4,00E+04		ND		detected
8,00E+04		ND		ND
1,00E+05		ND		ND

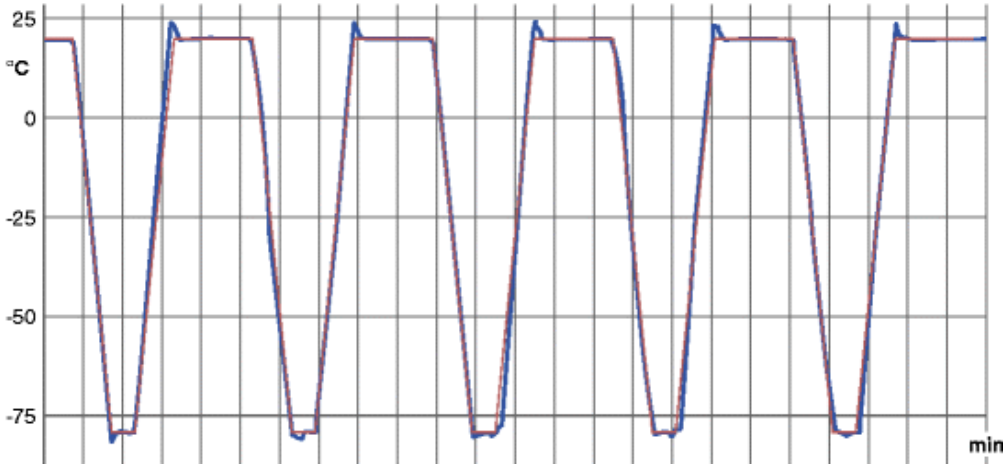
Dilution		Genexpert (influenza)		liat (influenza)
			Ct	
pure	influenza B	detected	18,5	detected
10		non testé		detected
1,00E+02		non testé		detected
1,00E+03		non testé		detected
1,00E+04		non testé		detected
1,00E+05		detected	35,4	detected
1,00E+06		ND		detected
2,00E+06		ND		detected
4,00E+06		non testé		detected
8,00E+06		non testé		ND
1,00E+07		non testé		ND

Dilution		Genexpert (influenza)		liat (influenza)
			Ct	
pure	influenza B	detected	16	detected
10		non testé		detected
1,00E+02		non testé		detected
1,00E+03		non testé		detected
1,00E+04		non testé		detected
1,00E+05		non testé		detected
1,00E+06		detected	37,9	detected
2,00E+06		ND		detected
4,00E+06		ND		detected
8,00E+06		non testé		ND
1,00E+07		non testé		ND

⇨ Cobas Liat Influenza A/B : **40 x plus sensible** que Xpert® Flu/RSV XC

4. Discussion : Study drawback (3)

- Freeze-thaw cycles are associated with degradation of viral nucleic acid :
  - Influenza A : frozen vs fresh samples ➔ delay of 0.5 Ct using Cobas Liat®
  - Influenza B : frozen vs fresh samples ➔ delay of 1.2 Ct using Cobas Liat®
  - Multiple freeze-thaw cycles performed!



5. Conclusion

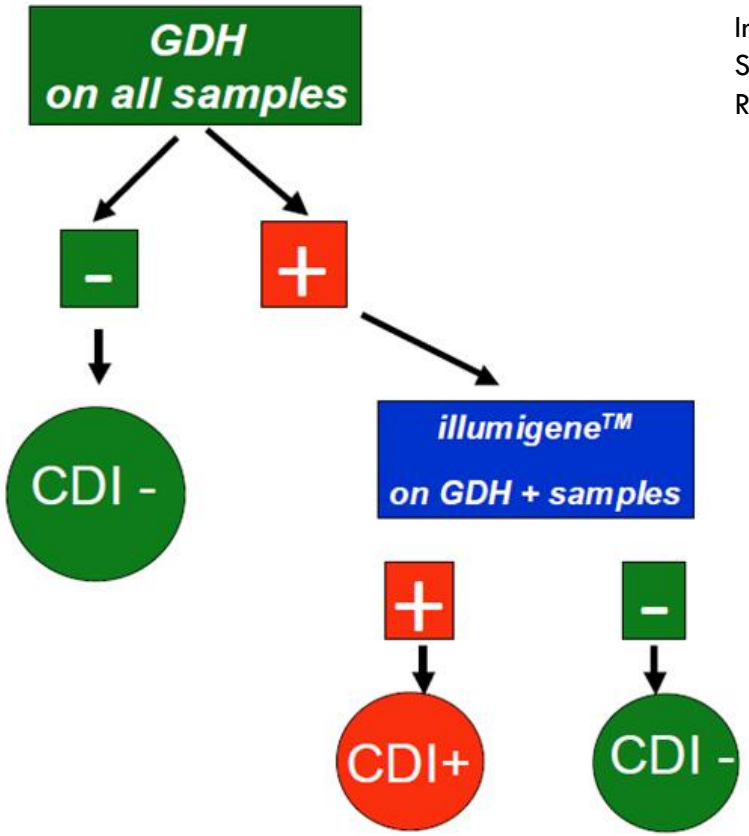
- Impact of viscosity (Cobas Liat®) and PBS (Liaison MDX®)
- Cobas Liat® sensitivity for influenza A, B and RSV of 100% and specificity of 89.8%, 100% and 87% respectively
- Liaison MDX® sensitivity for influenza A, B and RSV of 100%, 86.7% and 77.8 % and specificity of 100%, 95.5% and 100% respectively
- Study drawbacks :
  - Bias in the cohort (Alere i®)?
  - Reference method
  - Freeze-thaw cycles

2. Evaluation of the cobas®cdiff (Roche®) and the Simplexa® C. difficile Universal direct kit (Diasorin®) for the detection of toxigenic *Clostridium difficile* in routine stool samples

Online : Labmanager (26/10/2017)

Online : Roche (26/10/2017)

1. Introduction : screening strategy for detection of toxigenic *Clostridium difficile* at the lab



Immunocard® C. difficile GDH (Meridian)
Sensitivity = 97,5 %[a]
Results available in 30 minutes

Online : meridian.eu (28/09/17)

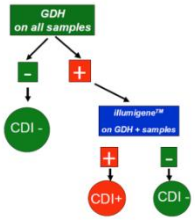
Online : meridian.eu (28/09/17)

Sensitivity = 73 %[b] ➔ 95,2 %[a]
Results available in 40 minutes

Van Broeck et Delmée, poster presentation, ECCMID London 2012, Poster n°2259

[a]According to manufacturer
[b]Walkty et al., 2013

1. Introduction : clinical performance of the GDH-Illumigen algorithm



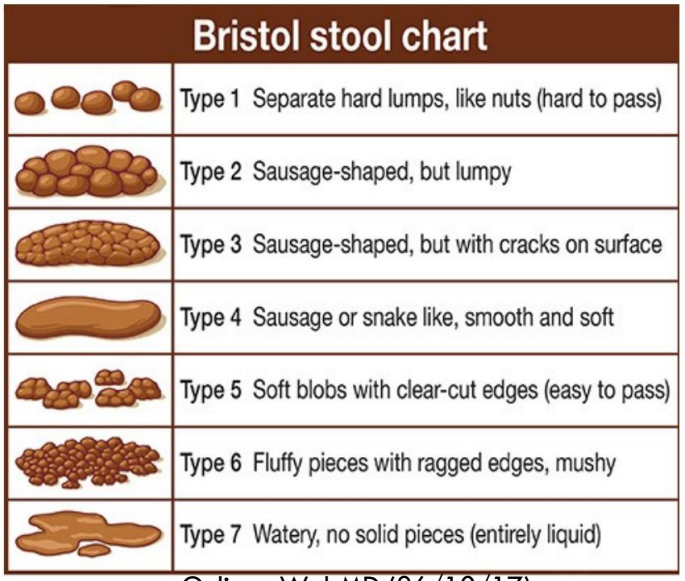
Study	Nbr of samples	Nbr of positive	GDH technique	Reference method	Sensitivity	Specificity
Van Broeck et Delmée, 2012	296	21 (7,1%)	• Premier® C. diff. GDH (Meridian®) • C. diff. Quick Check Complete® (Alere®)	Toxigenic Culture	81 %	91,6%
Eckert et al., 2014	308	• TC : 36 (11,7%) • CTA:23 (7,5%)	C. diff. Quick Check (Alere®)	• Toxigenic culture (TC) • Cytotoxicity assay (CTA)	• 86,1% (TC) • 95,7% (CTA)	• 99,6 % (TC) • 96,5 (CTA)
Walkty et al., 2013	428	63 (14,7%)	C. diff. Quick Check (Alere®)	Toxigenic culture	68,3 %	100 %

2. Material and Method : study design

- Single-center prospective study conducted in September 2017 at the « Hôpital Civil Marie Curie »

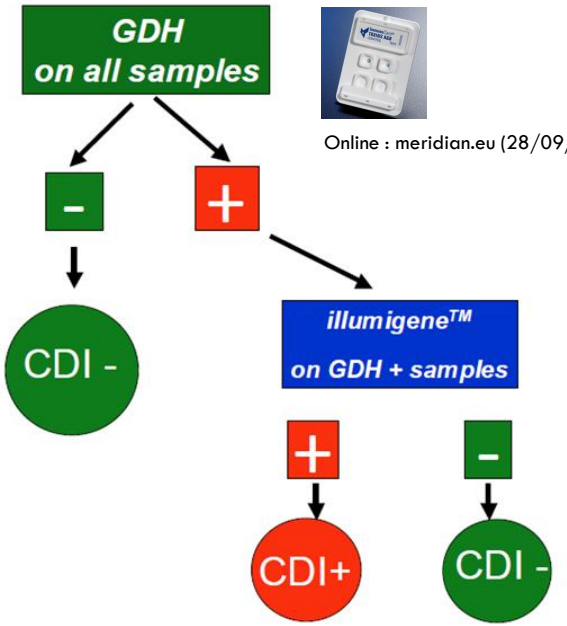
- Inclusion criteria :
  - Inpatients suffering from treatment-related diarrhea
  - Performed in the case of a specific request from the physician

- Exclusion criteria :
  - Bristol Scale ≤ 4 (formed stool)
  - In patients ≤ 2 years of age
  - Outpatients



Online : WebMD (26/10/17)

2. Material and Method : study design



Online : meridian.eu (28/09/17)

Van Broeck et Delmée, poster presentation, ECCMID London 2012, Poster n°2259

VS

Online : Roche (26/10/2017)

Online : Labmanager (26/10/2017)

Online : meridian.eu (28/09/17)

Discordant results ➔ Reference Laboratory (Belgium)

2. Material and Method : Timing!

- Two-step algorithm :
  - Fresh stool samples
  - GDH screening : 25 - 30 minutes
  - Illumigene® : 40 minutes (batch!, 2 X 5 samples MAX)
- Roche Liat®:
  - (sample transfer to UTM: 20 secondes)
  - Run configuration : 1 minute
  - Analysis: 20 minutes

Online : Roche (27/10/17)

- Laison MDX®:
  - (sample transfer to sample prep buffer vial: 20 secondes)
  - Run configuration : 1 minute/sample (batch!)
  - Analysis : 60-70 minutes (8 samples MAX)

Online : Diasorin (27/10/17)

3. Results

- Demographic data :
  - 53 stool samples
  - 38 F / 15 M
  - Mean age = 65 years old (SD 20,4 years)
  - Disease prevalence = 11,3 % (6 positive / 47 negative)

- Clinical performance :

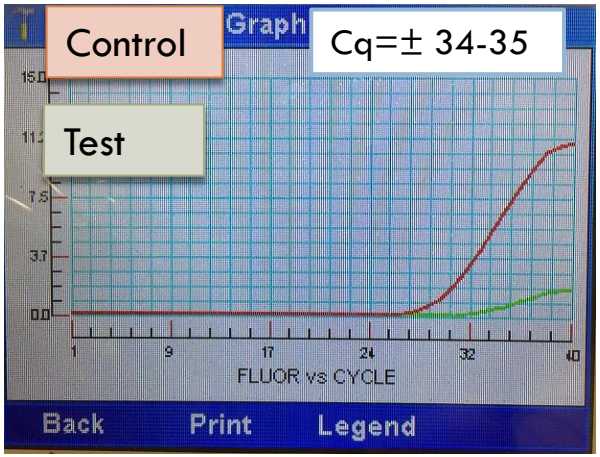
	invalid	sensibility	Specificity	PPV[a]	NPV[b]
Liat®	0	100 (54-100)	97,87 (88-99)	85,71 (46-97)	100 (91-100)
GDH-Illumigene® algorithm	1[c]	83 (42-99)	100 (90-100)	100 (51-100)	97,92 (87-99)
Illumigene®	1[c]	100 (54-100)	100 (90-100)	100 (51-100)	100 (91-100)
Liaison MDX®	0	100 (54-100)	97,87 (88-99)	85,71 (46-97)	100 (91-100)

[a]Positive Predictive Value. [b]Negative Predictive Value. [c]Same run

3. Discordant result

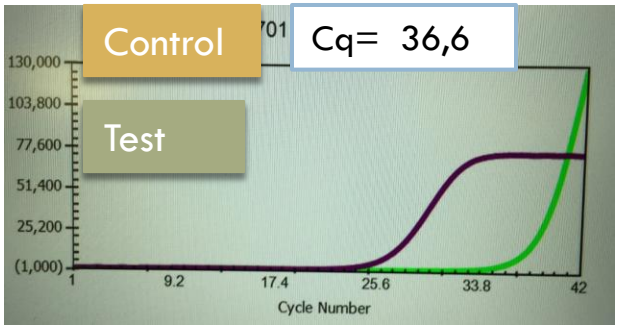
- Stool n° 50 :
  - Male, 58 y, Bristol scale of 6, GDH positive, Illumigen® negative

Liat® positive



Stool 50 Roche Liat®

Liaison MDX® positive



Stool 50 Liaison MDX®

  - National Reference Laboratory
    - GDH positive (Liaison® C. difficile GDH)
    - Toxine negative (Liaison ® C. difficile Toxins A & B)
    - Molecular method negative (GeneXpert®)

➔ Conclusion : CDI- (?)

Overdiagnosis ?

Original Investigation

Overdiagnosis of *Clostridium difficile* Infection in the Molecular Test Era

Christopher R. Polage, MD, MAS; Clare E. Gyorke, BS; Michael A. Kennedy, BS; Jhansi L. Leslie, BS; David L. Chin, PhD; Susan Wang, BS; Hien H. Nguyen, MD, MAS; Bin Huang, MD, PhD; Yi-Wei Tang, MD, PhD; Lenora W. Lee, MD; Kyoungmi Kim, PhD; Sandra Taylor, PhD; Patrick S. Romano, MD, MPH; Edward A. Panacek, MD, MPH; Parker B. Goodell, BS, MPH; Jay V. Solnick, MD, PhD; Stuart H. Cohen, MD

JAMA Intern Med. doi:10.1001/jamainternmed.2015.4114
Published online September 8, 2015.

Detection method :

- Toxin detection : C. difficile Premier Toxin A and B (Meridian®)
- Molecular methods : Xpert C. difficile/Epi (Cepheid®) and Illumigene (Meridian®) – Molecular results not reported !

N = 1416 : Tox+/PCR+ = 131; TOX-/PCR+ = 162; TOX-/PCR- = 1123

Outcomes :

- Duration of diarrrhea : TOX+/PCR+ > TOX-/PCR+ = TOX-/PCR-
- CDI-related complication: none in TOX-/PCR+ and TOX-/PCR- groups (10 cases in TOX+/PCR+ patients)
- CDI-related death : one in TOX-/PCR+ group vs 11 in TOX+/PCR+ group

4. Discussion : Comparison of clinical performance with other published data

1 Performance comparison of the cobas Liat and Cepheid GeneXpert systems for *Clostridium difficile* detection — N = 310

Paul A. Granato[1]*, Glen Hansen[2], Emily Herding[3], Sheena Chaudhuri[4], Shaowu Tang[4], Sachin K. Garg[4], Catherine R. Rowell[5], Joanna Jackson Sickler[4]

2 EQUIVALENT PERFORMANCE OF THE COBAS® CDIFF TEST FOR USE ON THE COBAS® LIAT® SYSTEM AND THE COBAS® 4800 SYSTEM — N = 442

Sachin K. Garg[1,*], Kyle Lu[2], John Duncan[1], Lance R. Peterson[3], Oliver Liesenfeld[1]

Sensitivity : 93.1 ➔ 100 % (100 %)
Specificity : 95.1 ➔ 100 % (97.87 %)

1 Comparative performance study of six commercial molecular assays for rapid detection of toxigenic *Clostridium difficile* — N = 210

Yossi Paitan, Tamar Miller Roll, Amos Adler

2 Comparison of Illumigene, Simplexa, and AmpliVue *Clostridium difficile* Molecular Assays for Diagnosis of *C. difficile* Infection — N = 200

E. Deak, S. A. Miller, R. M. Humphries

Sensitivity : 95 ➔ 98 % (100 %)
Specificity : 100 % (97.87 %)

5. Conclusion

- Timing !
  - Two-steps algorithm *vs* Roche Liat® or Liaison MDX®
  - Illumigene Meridian® *vs* Roche Liat® or Liaison MDX®
- Batch (Illumigne®, Liaison MDX®) *vs* « random access » (Roche liat®)
- Due to the lack of sensitivity of GDH screening, the two-step algorithm missed one case of CDI
- One false positive (?) for both Roche Liat® and Liaison MDX®
- Illumigene Meridian® scored a sensitivity and a specificity of 100%

3. Pros and Cons

Online : Labmanager (26/10/2017)

Online : Roche (26/10/2017)

Cobas Liat ® - Liaison MDX ®

Pros – Cobas Liat ®

- « Random Access »

  >< Liaison MDX® : bach

- Run configuration

  // Liaison MDX®

- Short run (20 minutes)

  >< Liaison MDX® : 60-70 minutes

- Robestness towards transport media composition

  >< Liaison MDX®

Cons – Cobas Liat ®

- Ct not presented

  >< Liaison MDX®

- Impact of viscosity

  >< Liaison MDX®

- Poor output

  >< Liaison MDX®

Cobas Liat ® *vs* Liaison MDX® output

M1-E-11292_20170712_00039-FRTA.rst

```
cobas Liat Result Report

[Report]
Assay:      Liat Influenza RSV Assay (FRTA)
Use:        For In vitro Diagnostic Use
Time/Date:  10:09:27, 2017-07-12
Sample ID:  1108144301
Report Results:
     Influenza A Detected
     Influenza B Not Detected
     RSV Not Detected
Details:
Run Status: OK
Device S/N: M1-E-11292
SW Ver:     3.0.0.2149
Run No. :   39
Tube S/N:   73EZ-02E51
Tube Lot:   70315Z
Tube Exp:   2018-03-31
Ctrl Exp:   N/A
Operator:   ADMIN
Approved By: ADMIN

[HLData]
MacAddress: F8:DC:7A:07:7A:8D
AnalysisDateTime: 2017-07-12T08:09:27Z
OrderingPhysician: N/A
SampleType: UTM
Patient:
Observation: Influenza A
Interpretation: Detected
Observation: Influenza B
Interpretation: Not Detected
Observation: RSV
Interpretation: Not Detected
```

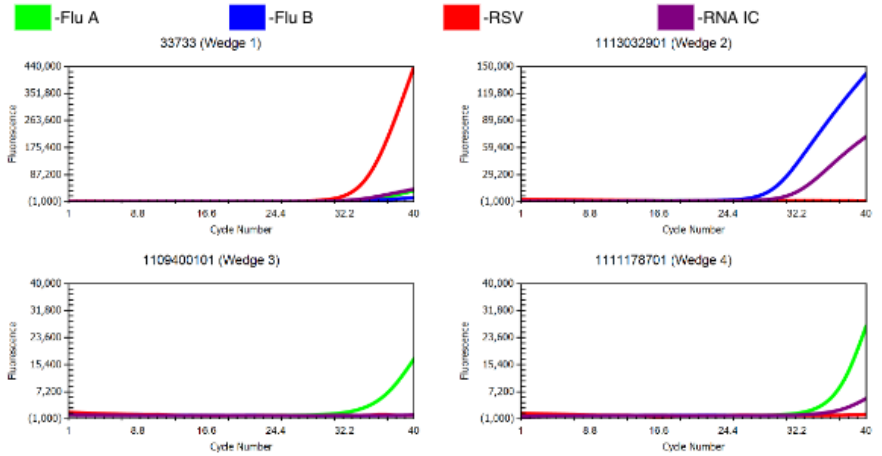
vs

Ct Values

Wedge	Sample Id	Sample Type	Flu A (FAM)	Flu B (JOE)	RSV (CFR610)	RNA IC (Q670)	QC Statement/Notes
1	33733	PC-FABR	33.5	35.1	31.1	33.2	
2	1113032901	Unknown	0	26.8	0	30.9	
3	1109400101	Unknown	34.9	0	0	0	
4	1111178701	Unknown	34.9	0	0	39.9	
5	1112551201	Unknown	0	0	0	35.5	
6	1111323701	Unknown	35.8	0	0	33.7	
7	1112985901	Unknown	35.4	0	0	36.2	
8	1112655301	Unknown	34.8	0	0	28.9	

Results

Wedge	Sample Id	Sample Type	Flu A (FAM)	Flu B (JOE)	RSV (CFR610)	RNA IC (Q670)	QC Statement/Notes
1	33733	PC-FABR	Detected	Detected	Detected	Valid	
2	1113032901	Unknown	Not Detected	Detected	Not Detected	Valid	
3	1109400101	Unknown	Detected	Not Detected	Not Detected	Valid	
4	1111178701	Unknown	Detected	Not Detected	Not Detected	Valid	
5	1112551201	Unknown	Not Detected	Not Detected	Not Detected	Valid	
6	1111323701	Unknown	Detected	Not Detected	Not Detected	Valid	
7	1112985901	Unknown	Detected	Not Detected	Not Detected	Valid	
8	1112655301	Unknown	Detected	Not Detected	Not Detected	Valid	



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