DIAGNOSTIC PERFORMANCE OF TRIPLEX NOROVIRUS/ROTAVIRUS/ADENOVIRUS ICG TESTS

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INTRODUCTION

- Norovirus (NoV), rotavirus (RVA) and adenovirus (AdV) are the principal viruses responsible for acute gastroenteritis (AGE) in humans.
- Recently, "triplex" immunochromatographic tests (ICG), allowing the rapid and simultaneous detection of these 3 pathogens in human stools, have been introduced to the French market.

OBJECTIVE

► To determine the diagnostic performance of 4 ICG tests available in France for the rapid and simultaneous detection of norovirus, rotavirus and adenovirus.

MATERIALS AND METHODS

STOOL SAMPLES

- Crude fecal material collected between 2000 and 2018 from patients suffering from AGE (storage at -40°C)
- Viral strains selected from among the most epidemiologically important viral strains in France
- **157 samples included**, among them 11 samples with co-infection (8 RVA + AdV, 3 RVA + NoV) and 39 samples negative for the 3 viruses

NoV (n=40)		RVA (n=43)	AdV (n=46)
genogroup I (n=20)	genogroup II (n=20)		
4 GI.1	3 GII.2[P16]	10 G1P[8]	26 type F
4 GI.2	1 GII.3	8 G2P[4]	3 type B
4 GI.4	4 GII.4[P4]	5 G3P[8]	14 type C
4 GI.6[PNA1]	4 GII.4[P16]	5 G4P[8]	1 type D
4 GI.7	4 GII.6[P7]	7 G9P[8]	2 non typable
	4 GII.17	5 G12P[8]	
		3 G-UDP-UD	

ICG TESTS

► Parallel evaluation of the following 4 triplex ICG tests (July to August 2018):

- **bioNexia® Noro/Rota-Adeno** (product code 415089, bioMérieux)
- **IMMUNOQUICK® NoRotAdeno** (product code 1150013, Biosynex)
- **ROTA+ADENO+NORO Combo Card** (product code RA872001V, CerTest Biotec)
- **RIDA®QUCIK Rota/Adeno/Noro Combi** (product code N1903, R-Biopharm)

REFERENCE METHODOLOGY

- NoV and RVA: Real-time RT-PCR in real time adapted from the literature by the CNR (National Research Council) *[Ouédraogo et al., PLoS One 2016;11(4):e0153652]*
- AdV: Commercial real-time PCR *(Adenovirus R-gene®, bioMérieux)*
► Reference methodology repeated on the same day as the ICG's in the following cases:
  - testing for presence of the virus of interest when all the ICG's show a negative result on an a priori positive sample
  - testing for absence of virus when at least one ICG shows a positive result on a sample which was *a priori* negative for this virus

RESULTS

► NOROVIRUS (Fig. 1)

❖ **Overall sensitivity: 42.5%** (CI95%: 27.0-59.1%) **to 87.5%** (CI95%: 73.2-95.8%)
  ➢ high sensitivity of the R-Biopharm test but poor sensitivity for the other 3 ICG's
  ➢ genogroup-dependent performance characteristics:
    - NoV GI: high sensitivity for R-Biopharm (90%) poor sensitivity for the other 3 ICG's (10-30%)
    - NoV GII: fairly high sensitivity (65-85%) similar performance characteristics for the 4 ICG's

❖ **Specificity: 96.6%** (CI95%: 91.5-99.1) **to 99.1%** (CI95%: 95.3-100)
  ➢ excellent specificity and similar performance characteristics for the 4 ICG's

Note: NoV GII is the most significant in terms of epidemiology (72-93% of AGE epidemics in France in the last 5 years). The sample used for this study is not representative of the distribution of NoV genogroups in the population.

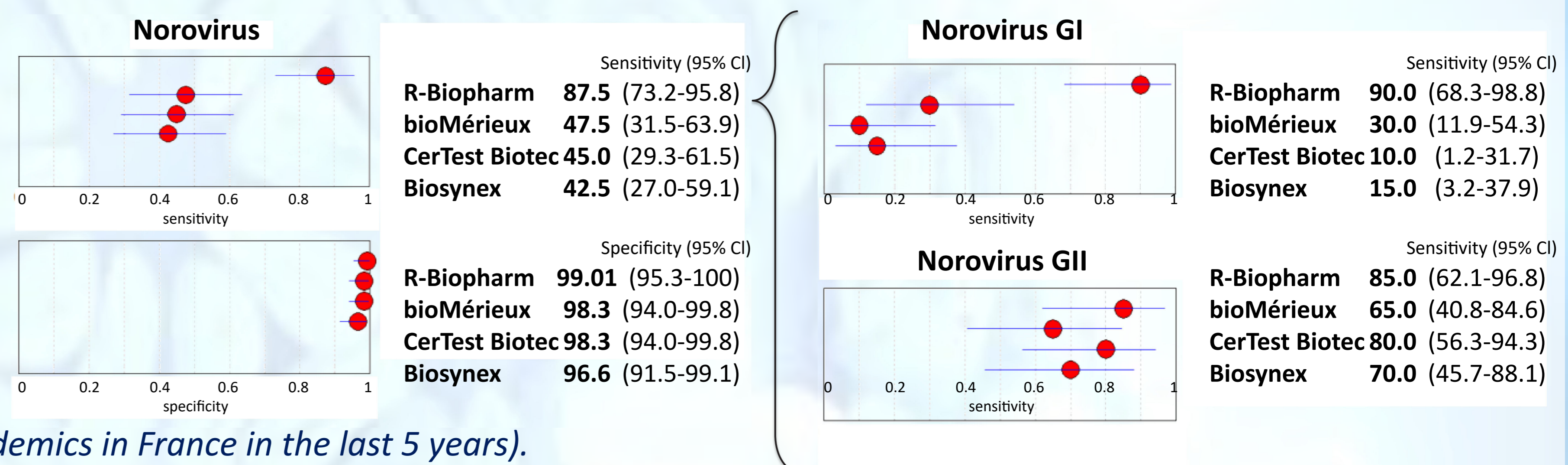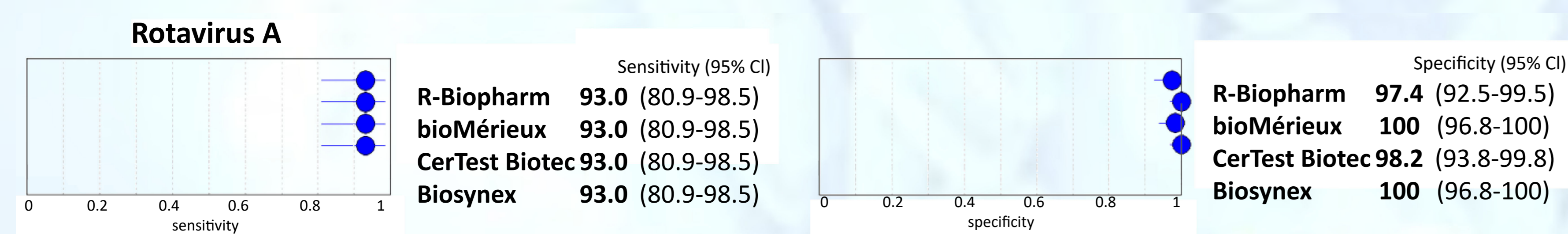
Fig. 1 – Norovirus ICG sensitivity and specificity

Norovirus	Sensitivity (95% CI)
R-Biopharm	87.5 (73.2-95.8)
bioMérieux	47.5 (31.5-63.9)
CerTest Biotec	45.0 (29.3-61.5)
Biosynex	42.5 (27.0-59.1)

	Specificity (95% CI)
R-Biopharm	99.01 (95.3-100)
bioMérieux	98.3 (94.0-99.8)
CerTest Biotec	98.3 (94.0-99.8)
Biosynex	96.6 (91.5-99.1)

Norovirus GI	Sensitivity (95% CI)
R-Biopharm	90.0 (68.3-98.8)
bioMérieux	30.0 (11.9-54.3)
CerTest Biotec	10.0 (1.2-31.7)
Biosynex	15.0 (3.2-37.9)

Norovirus GII	Sensitivity (95% CI)
R-Biopharm	85.0 (62.1-96.8)
bioMérieux	65.0 (40.8-84.6)
CerTest Biotec	80.0 (56.3-94.3)
Biosynex	70.0 (45.7-88.1)

► ROTAVIRUS (Fig. 2)

❖ **Sensitivity: 93.0%** (CI95%: 80.9-98.5%)
  ➢ high sensitivity and similar performance characteristics for the 4 ICG's

❖ **Specificity: 97.4%** (CI95%: 92.5-99.5%) **to 100%** (CI95%: 96.8-100%)
  ➢ excellent specificity and similar performance characteristics for the 4 ICG's

Fig. 2 – Rotavirus ICG sensitivity and specificity

Rotavirus A	Sensitivity (95% CI)
R-Biopharm	93.0 (80.9-98.5)
bioMérieux	93.0 (80.9-98.5)
CerTest Biotec	93.0 (80.9-98.5)
Biosynex	93.0 (80.9-98.5)

	Specificity (95% CI)
R-Biopharm	97.4 (92.5-99.5)
bioMérieux	100 (96.8-100)
CerTest Biotec	98.2 (93.8-99.8)
Biosynex	100 (96.8-100)

► ADENOVIRUS (Fig. 3)

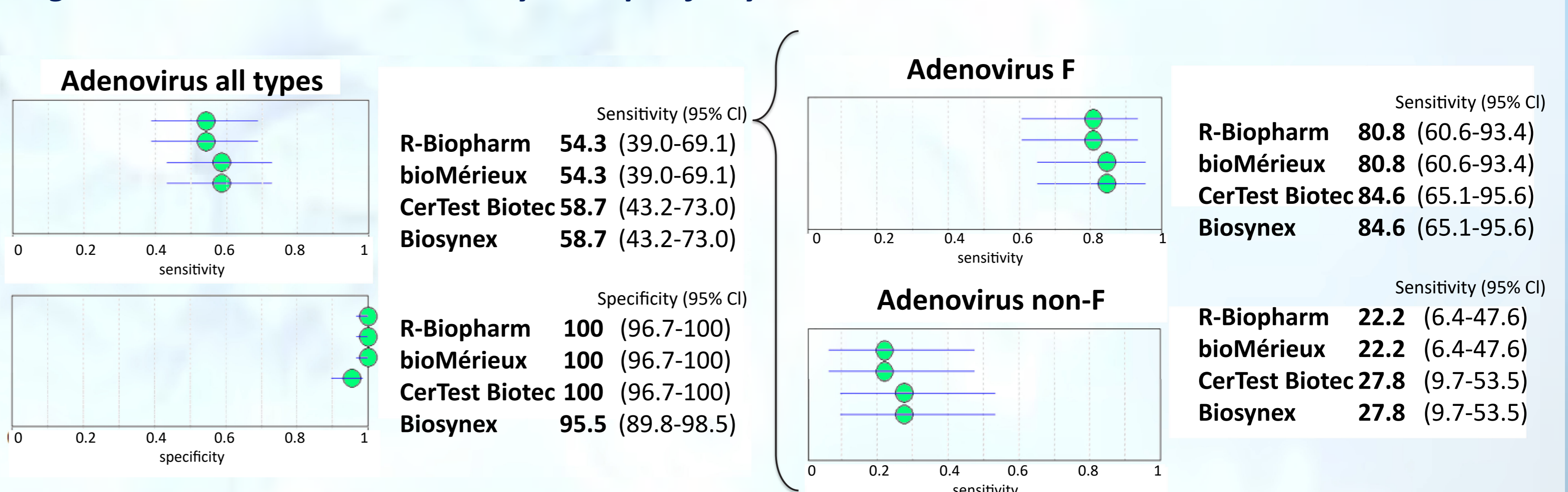❖ **Overall sensitivity: 54.3%** (CI95%: 39.0-69.1%) **to 58.7%** (CI95%: 43.2-73.0%)
  ➢ poor sensitivity and similar performance characteristics for the 4 ICG's
  ➢ better performance characteristics with type F (80.8-84.6%) than with the other types (22.2-27.8%)

❖ **Specificity: 95.5%** (CI95%: 89.8-98.5%) **to 100%** (CI95%: 96.7-100%)
  ➢ excellent specificity, similar performance characteristics for the 4 ICG's

Note: type F (types 40/41) is the most important in terms of epidemiology in the context of AGE.

Fig. 3 – Adenovirus ICG sensitivity and specificity

Adenovirus all types	Sensitivity (95% CI)
R-Biopharm	54.3 (39.0-69.1)
bioMérieux	54.3 (39.0-69.1)
CerTest Biotec	58.7 (43.2-73.0)
Biosynex	58.7 (43.2-73.0)

	Specificity (95% CI)
R-Biopharm	100 (96.7-100)
bioMérieux	100 (96.7-100)
CerTest Biotec	100 (96.7-100)
Biosynex	95.5 (89.8-98.5)

Adenovirus F	Sensitivity (95% CI)
R-Biopharm	80.8 (60.6-93.4)
bioMérieux	80.8 (60.6-93.4)
CerTest Biotec	84.6 (65.1-95.6)
Biosynex	84.6 (65.1-95.6)

Adenovirus non-F	Sensitivity (95% CI)
R-Biopharm	22.2 (6.4-47.6)
bioMérieux	22.2 (6.4-47.6)
CerTest Biotec	27.8 (9.7-53.5)
Biosynex	27.8 (9.7-53.5)

CONCLUSION

► **Used in first line testing, the 4 ICG "triplex" tests are suitable for:**
  - rapid detection of NoV GII, the most important genogroup in terms of epidemiology, while only a single ICG test is suitable for the rapid detection of NoV GI in human stool
  - the rapid diagnosis of RVA infections in human stool
  - the rapid diagnosis of AdV, but only in the context of AGEs in which the primary agent is AdV F (types 40/41). The risk of not detecting certain non-F AdVs also associated with AGE should be borne in mind when using these tests.

► Because of their superior diagnostic performance characteristics, especially in terms of sensitivity, **molecular biology techniques remain the "gold standard" for the diagnosis of viral AGEs.**

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