

PCR multiplexe syndromique : Principe, bonnes pratiques et application clinique

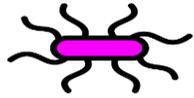
Pr Florence DOUCET-POPULAIRE

université
PARIS-SACLAY

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Antoine-Béclère
AP-HP



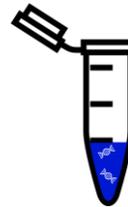
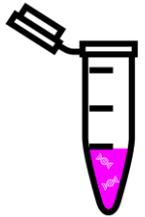
PCR MULTIPLEX : Innovation et révolution en microbiologie



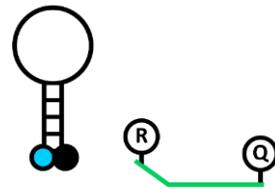
Pathogène 1



Gène de Résistance



Détection : SONDÉS

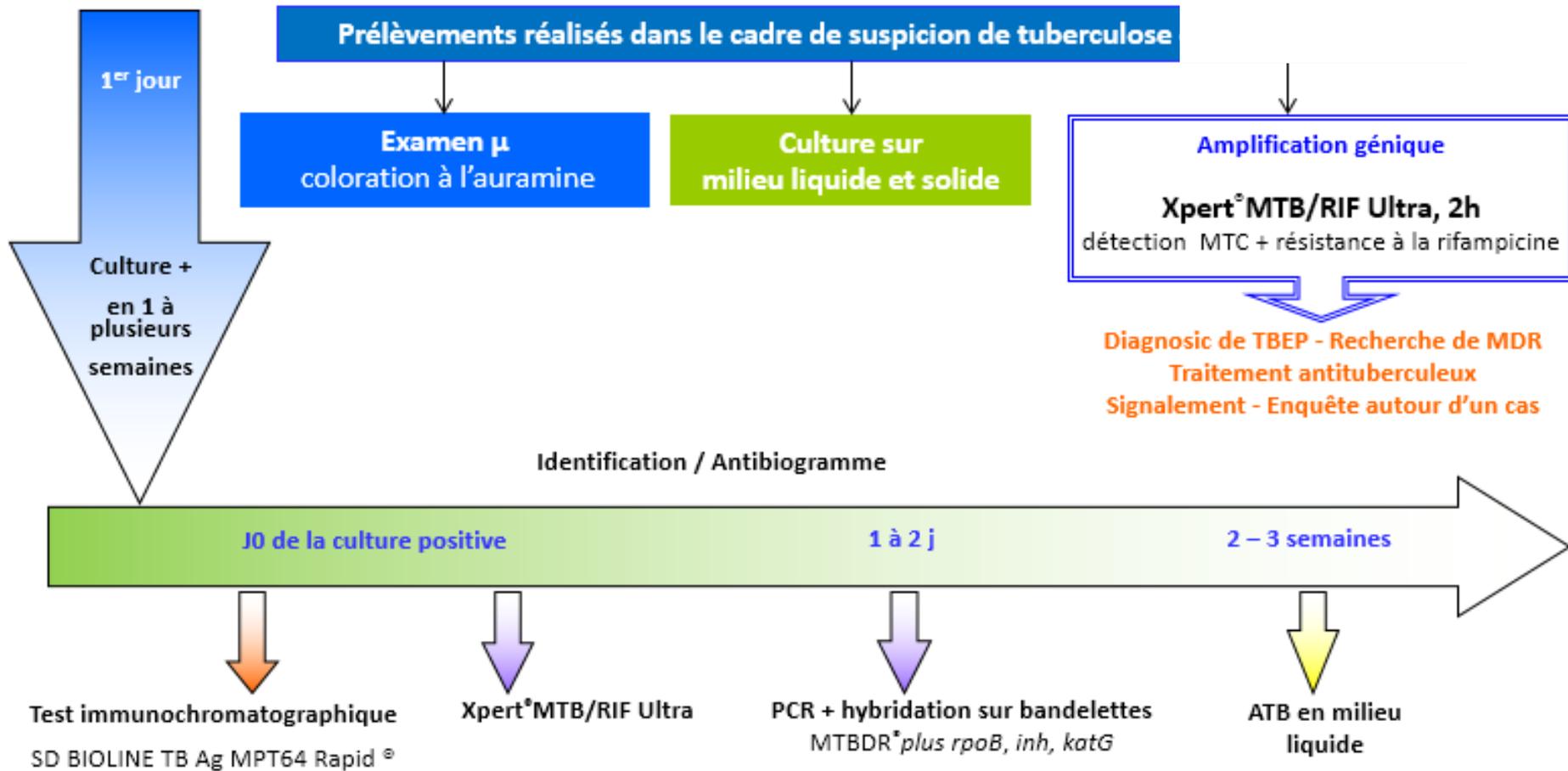


ETAPES

1. Purification des Ac. Nucléiques
2. Reverse transcription si nécessaire
3. PCR Multiplex
4. Détection
5. **Validation**
6. **Interprétation**

Diagnostic Rapide et au plus près du patient

Prise en charge de la tuberculose en prison



Improving tuberculosis management in prisons: Impact of a rapid molecular point-of-care test

Marine Evrein^{a,1}, Loïc Hermet^{b,1}, Christelle Guillet-Caruba^a, Pierre-Louis Nivose^b, Vallier Sordoillet^a, Guillaume Mellon^b, Anne Dulioust^b, Florence Doucet-Populaire^{a,c,*}

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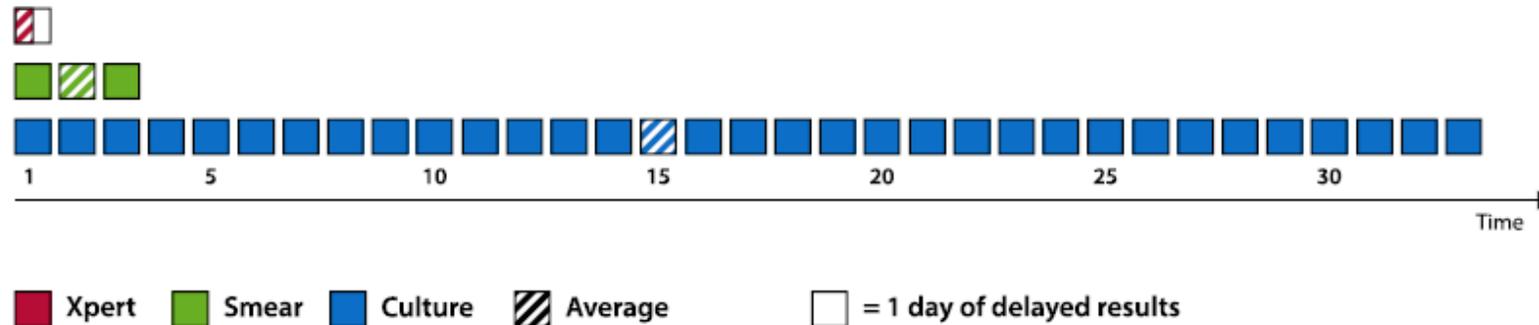
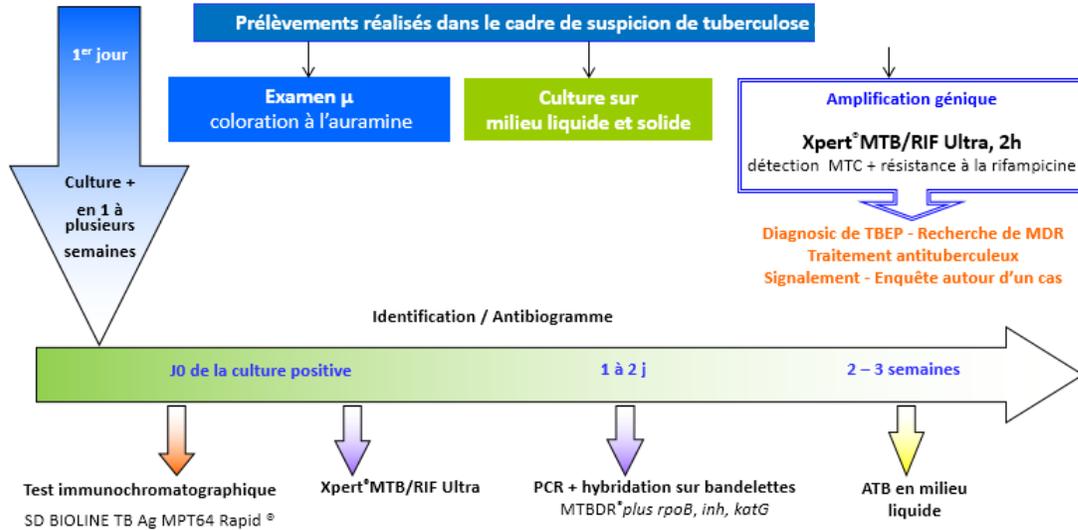


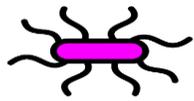
Table 3

Xpert[®]MTB/RIF Ultra sensitivity and specificity on the raw and decontaminated sputum.

Xpert sensibility and specificity	Smear positive	Smear negative	All samples
Raw sputum (n = 94)			
Sensitivity	100%	85.7%	92.3%
	95% CI: 61 - 100%	95% CI: 48.7 - 97.4%	95% CI: 66.7 - 98.6%
Specificity			100%
			95% CI: 95.3 - 100%
Decontaminated sputum (n = 200)			
Sensitivity	100%	76.9%	85.7%
	95% CI: 67.6 - 100%	95% CI: 49.7- 91.8%	95% CI: 65.4 - 95%
Specificity			100%
			95% CI: 97.9 - 100%



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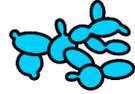
Pathogène 1



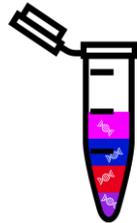
Pathogène 2



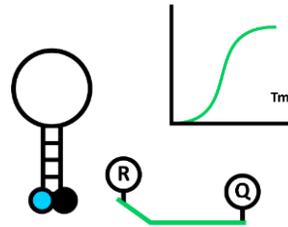
Pathogène 3



Pathogène 4



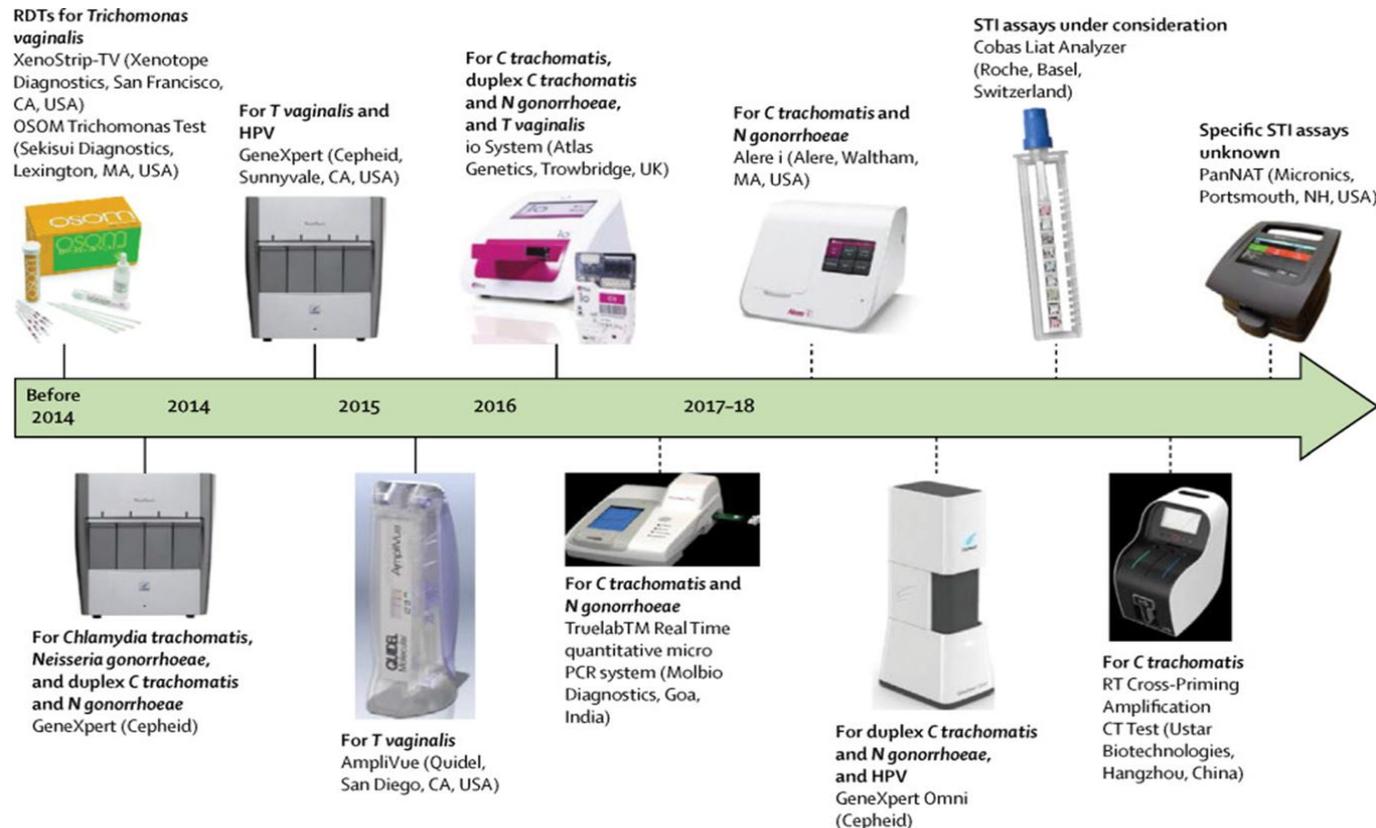
Détection : SONDÉS - Tm



- Diagnostic Rapide
- au plus près du patient
- Dépister et Traiter

de La PCR simplex à la PCR multiplex Exemple des Infections Sexuellement transmissibles

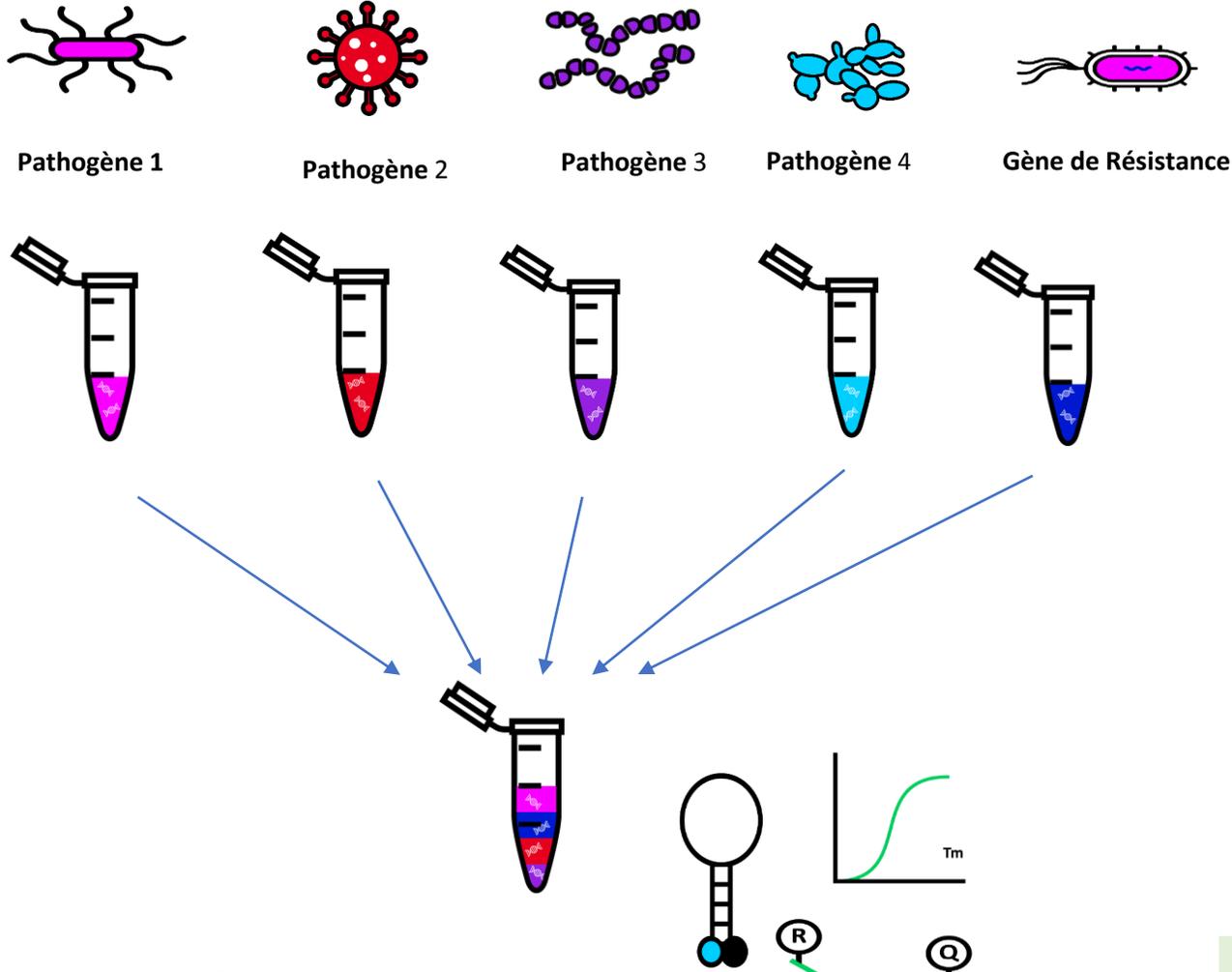
- Un million de personnes infectées chaque jour par l'une des quatre IST curables : *C. trachomatis*, *N. gonorrhoeae*, syphilis et *Trichomonas*



+ Dépister et traiter

- Détection uniquement des pathogènes prédéfinis

Tests de POC ou « near POC » Pour le diagnostic des STI

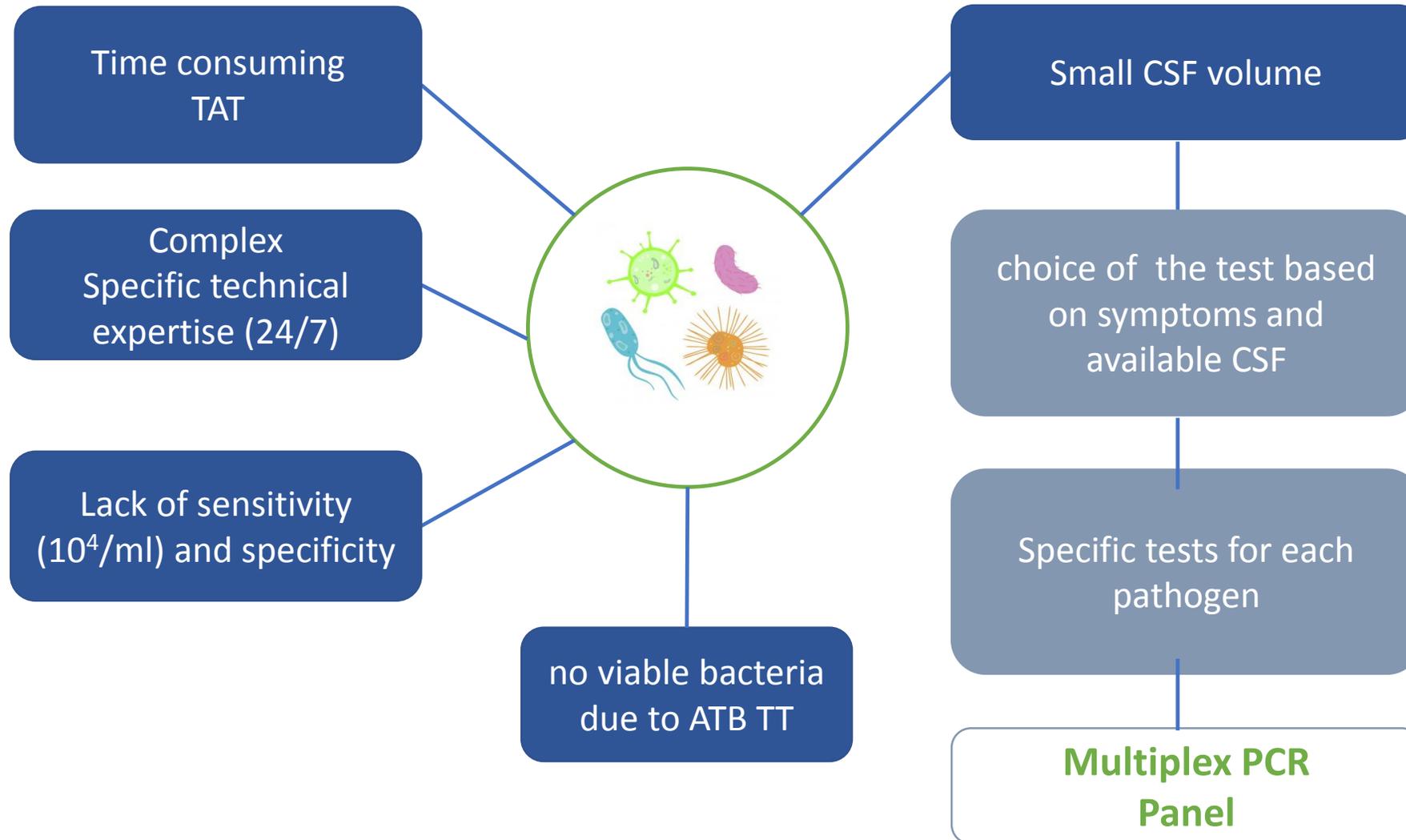


Approche syndromique

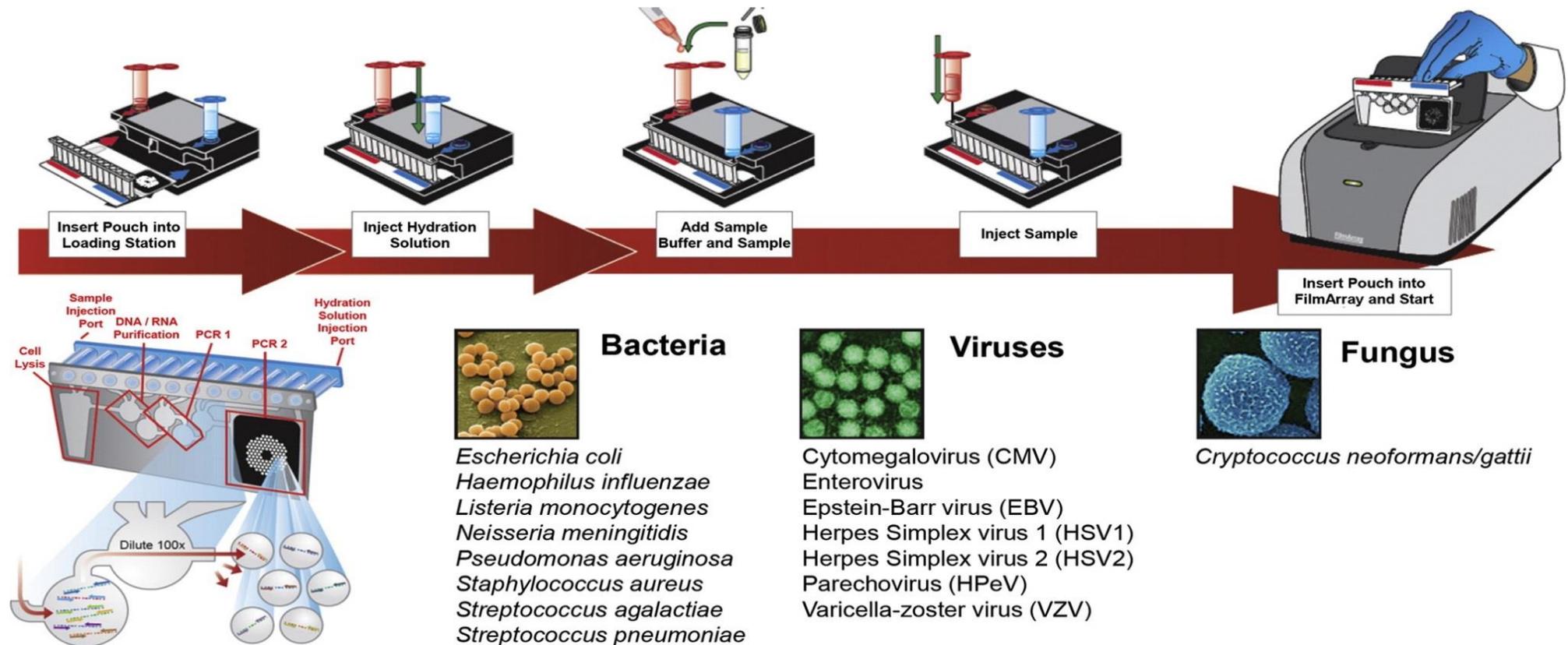
- **1** syndrome (plusieurs symptômes) pour multiples étiologies microbiologiques
- 1 seul prélèvement et 1 seul test
- Délai exécution rapide
- Interprétation des tests

Meilleure prise en charge du patient
Bon usage des anti-infectieux

Limites du diagnostic des méningites bactériennes



Menigitis/encephalitis panel (FilmArray®)



Joshua Rhein et al . Diagnostic Microbiology and Infectious Disease, Volume 84, 2016, 268–273

2 minutes of hands-on-time ; TAT 1 hour

TABLE 2. Studies of the BioFire FilmArray ME Panel Included in the 2019 Meta-analysis

Study	Study Population	Total No. of Samples in the Study	Type of Study	Overall Sensitivity	Overall Specificity	PPV	NPV
Leber et al ²⁵	Pediatrics and adults	1560	Prospective	94.2%	97.7%	74.8%	99.6%
Arora et al ²⁶	Pediatrics	62	Prospective	100.0%	93.4%	55.6%	100.0%
Lee et al ²⁷	Pediatrics and adults	42	Prospective	60.0%	100%	100.0%	86.7%
Radmard et al ²⁴	Pediatrics and adults	705	Retrospective	85.7%	98.3%	36.8%	99.9%
Hanson et al ²⁸	Pediatrics and adults	342	Retrospective	91.8%	88.3%	88.4%	91.7%
Messacar et al ²⁹	Pediatrics	138	Retrospective	91.1%	97.9%	95.3%	95.9%
Graf et al ³⁰	Pediatrics	133	Retrospective	92.5%	100.0%	100.0%	93.0%
Piccirilli et al ³¹	Pediatrics and adults	63	Retrospective	85.7%	100.0%	100.0%	77.8%

Fleischer et Aronson ; *Pediatr Emerg Care.* 2020 Aug; 36(8): 397–401.

Avantages et Limites

Potential Benefits

- Faster turnaround time, diagnosis, and definitive treatment/treatment discontinuation^{30,37,38}
- Pathogen identification in culture-negative CSF samples from patients with suspected bacterial meningitis^{38,43–46}
- Detection of organisms in CSF obtained after antimicrobial pretreatment^{38,43,47–49}
- Enables simultaneous identification of coinfections on the same sample^{9,50}
- Ability to test for multiple organisms simultaneously²⁴
- Facilitates proper administration of chemoprophylaxis for close contacts⁴⁹
- Relatively small amount of CSF sample (minimum 0.2 mL) required²⁴
- Limited hands-on time and technical expertise necessary^{14,27}

Potential Limitations

- Concern for false-positive and false-negative tests^{14,17,28,33,51–53}
- Not all pathogens able to cause CNS infections are detected by the panel^{24,26,31,33,37,38,53,54}
- Unable to provide antimicrobial susceptibilities^{24,33}
- Not intended for CSF samples obtained from indwelling CNS medical devices²⁴
- Positive results do not exclude the possibility of a coinfection with an organism not in the panel²⁴
- Relatively high cost of purchase (\$35,550–\$50,000), service (\$4000/y) and per test (~\$200)^{13,27,55}
- Lower ability to detect viruses when compared with some singleplex assays^{17,40,50,56}
- Positive results for herpesviruses may be due to latency or reactivation of the virus with or without disease^{17,33}

Fleischer et Aronson ; Pediatr Emerg Care. 2020 Aug; 36(8): 397–401.

Approche syndromique-CONCLUSION

- ✓ Diagnostic rapide qui facilite la prise en charge des infections
- ✓ **Importance de l'interprétation post-analytique** en fonction de la clinique mais aussi des résultats des autres examens biologiques
- ✓ **On ne trouve que ce que l'on cherche**
- ✓ Le panel final commercialisé ne répondra jamais aux besoins de tous les laboratoires
- ✓ Rapport coût/bénéfice
- ✓ Place dans l'algorithme diagnostic
- ✓ Stratégie d'utilisation optimale à définir