La place des approches moléculaires dans le diagnostic de la tuberculose

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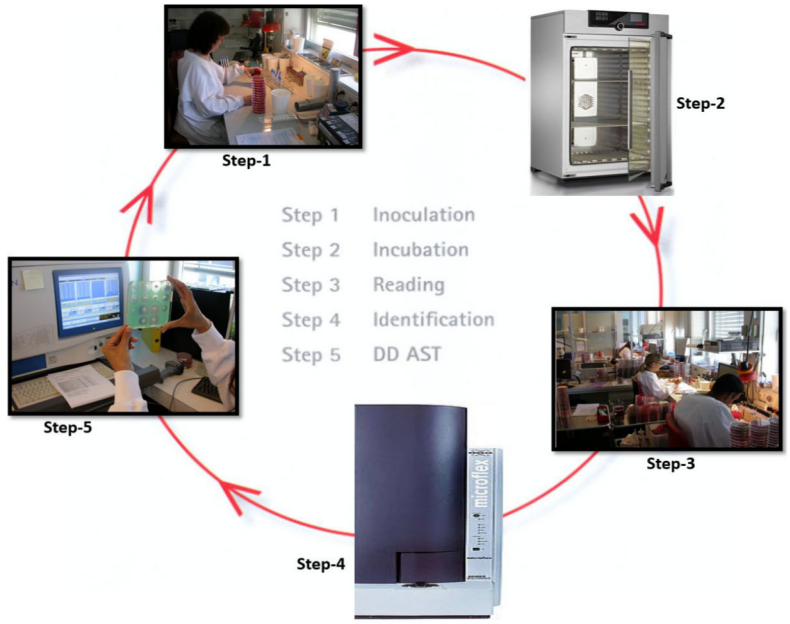
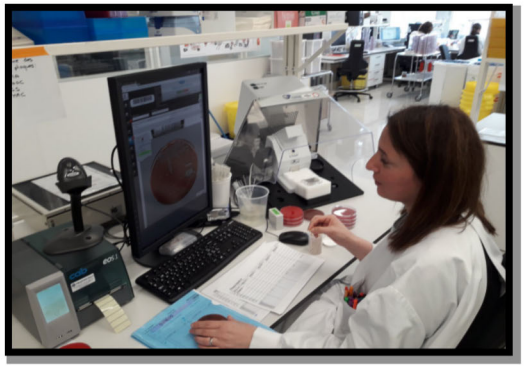
Journées scientifiques du ccCTA
15 Septembre 2023

Introduction

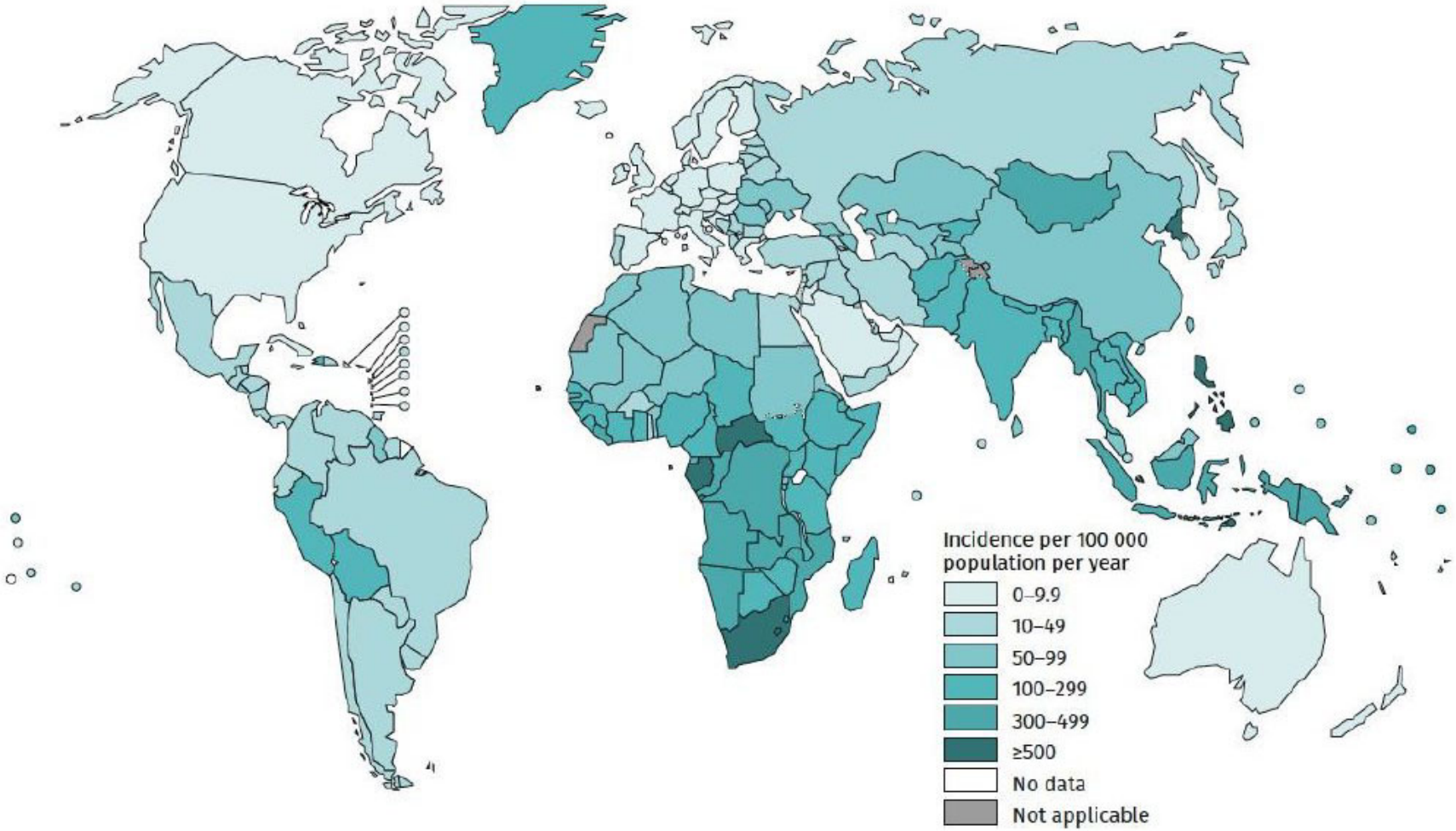

Step-1
Step-2
Step 1 Inoculation
Step 2 Incubation
Step 3 Reading
Step 4 Identification
Step 5 DD AST
Step-5
Step-3
Step-4


WASPLab

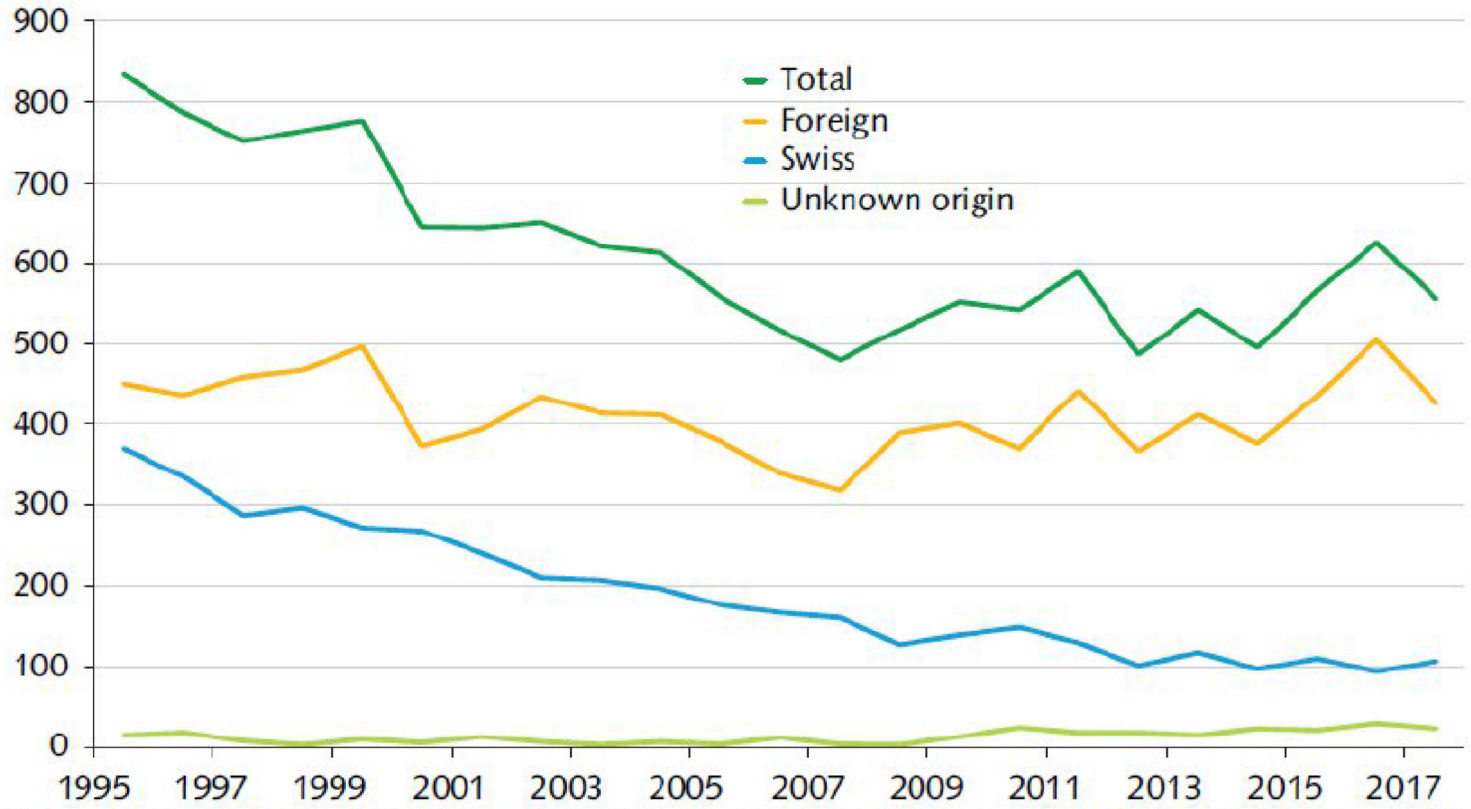


Epidémiologie

Estimated TB incidence rates, 2020



En Suisse, le nombre de cas déclarés en 2017 était de 554, (6,5 nouveaux cas pour 100'000 personnes). Parmi ceux-ci, 77% étaient des personnes d'origine étrangère



Cas de tuberculose déclarés à l'Office fédéral de la santé publique selon l'origine, Suisse, 1995 - 2017

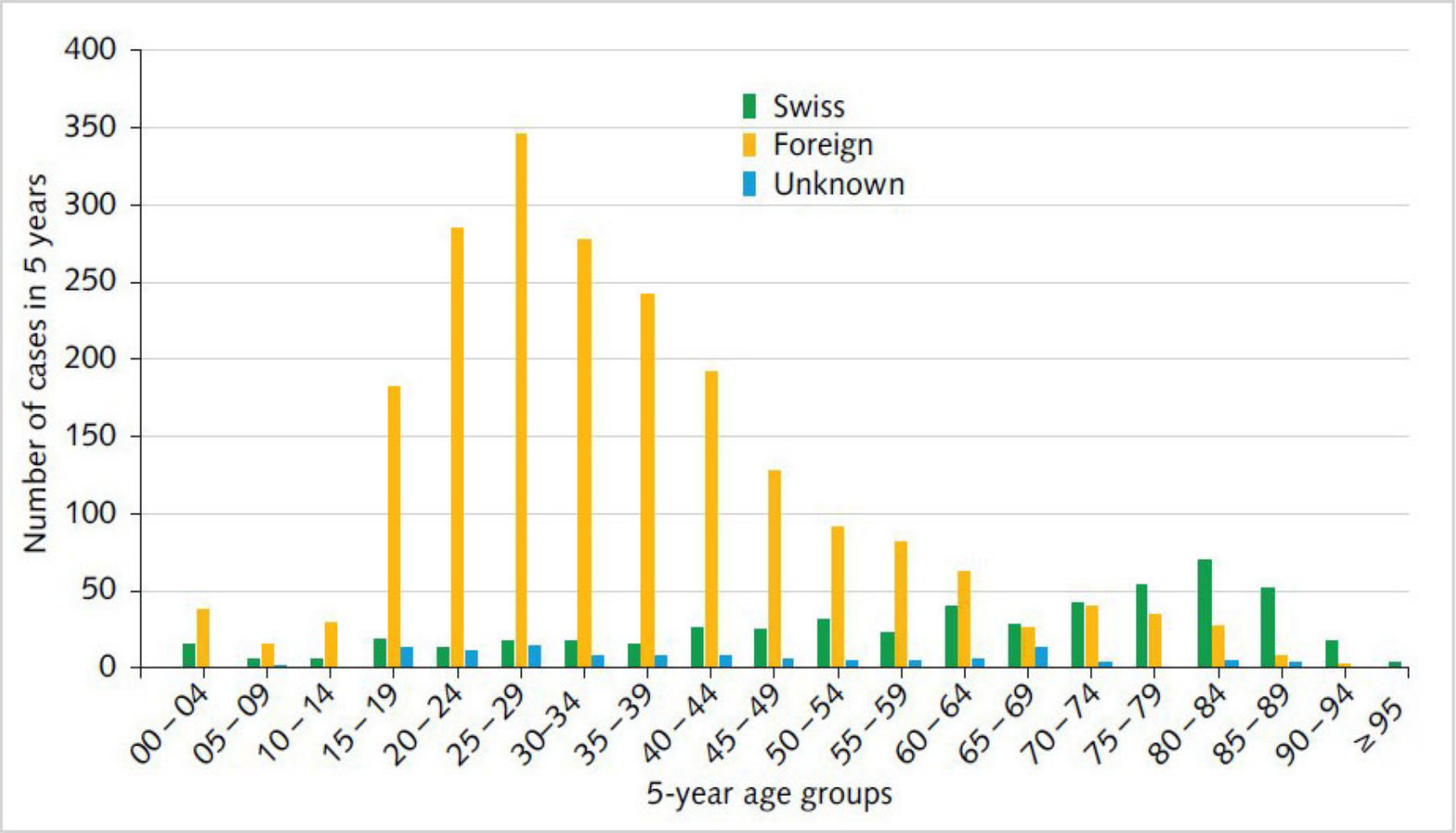
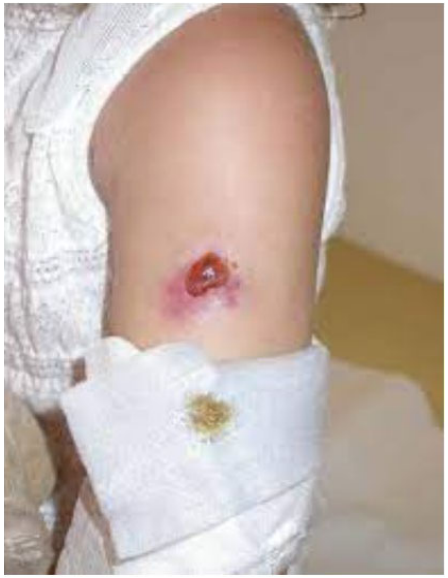


Figure 2-2. Ages et origines des cas déclarés à l'Office fédéral de la santé publique, Suisse, de 2012 à 2016 (n total=2716).

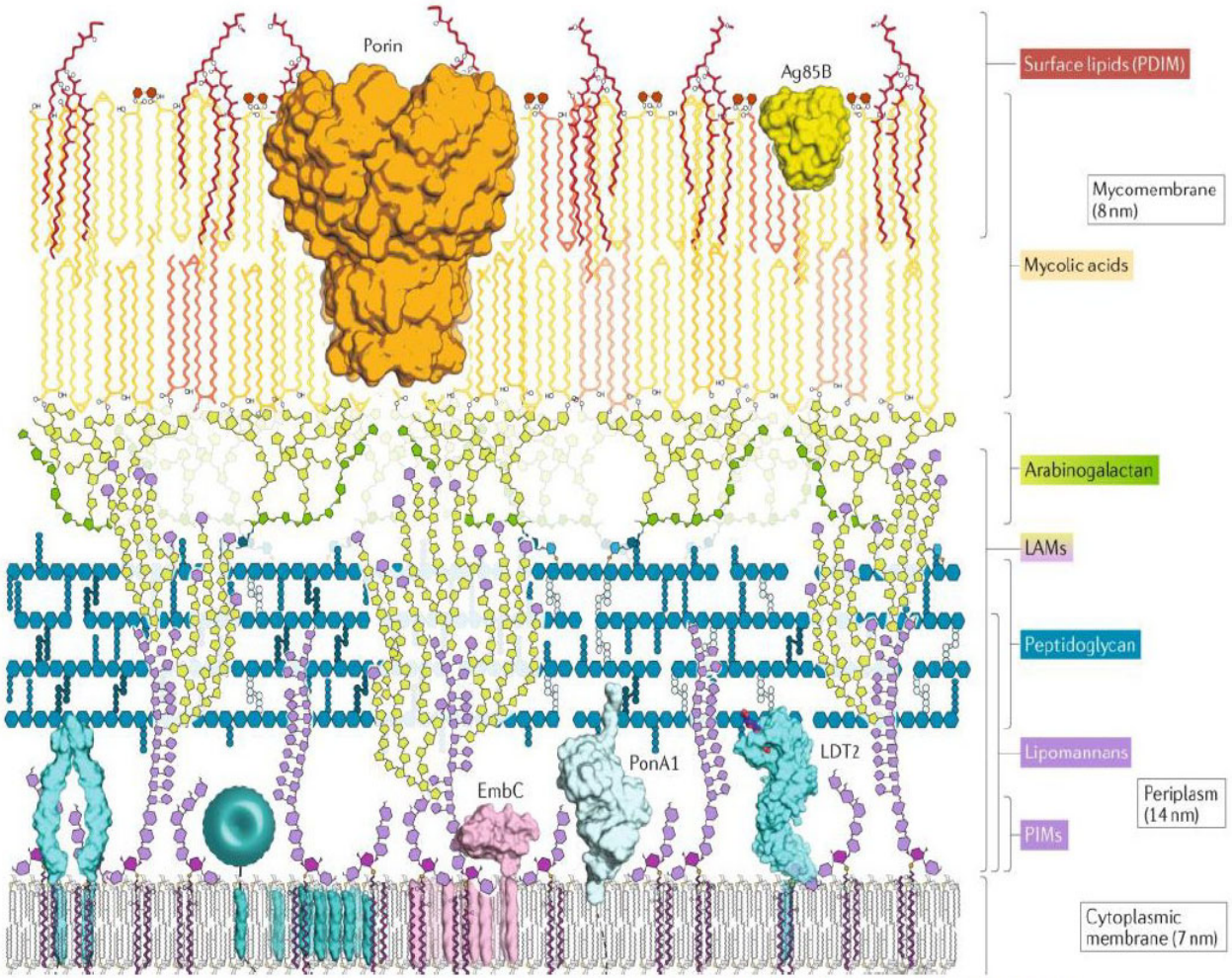
"The TB vaccine is called **Bacille Calmette-Guérin (BCG)**, and it is used in many countries to prevent severe forms of TB in children. **However, BCG is not generally recommended in the United States because it has limited effectiveness for preventing the most common forms of TB and in preventing TB in adults."** **CDC**



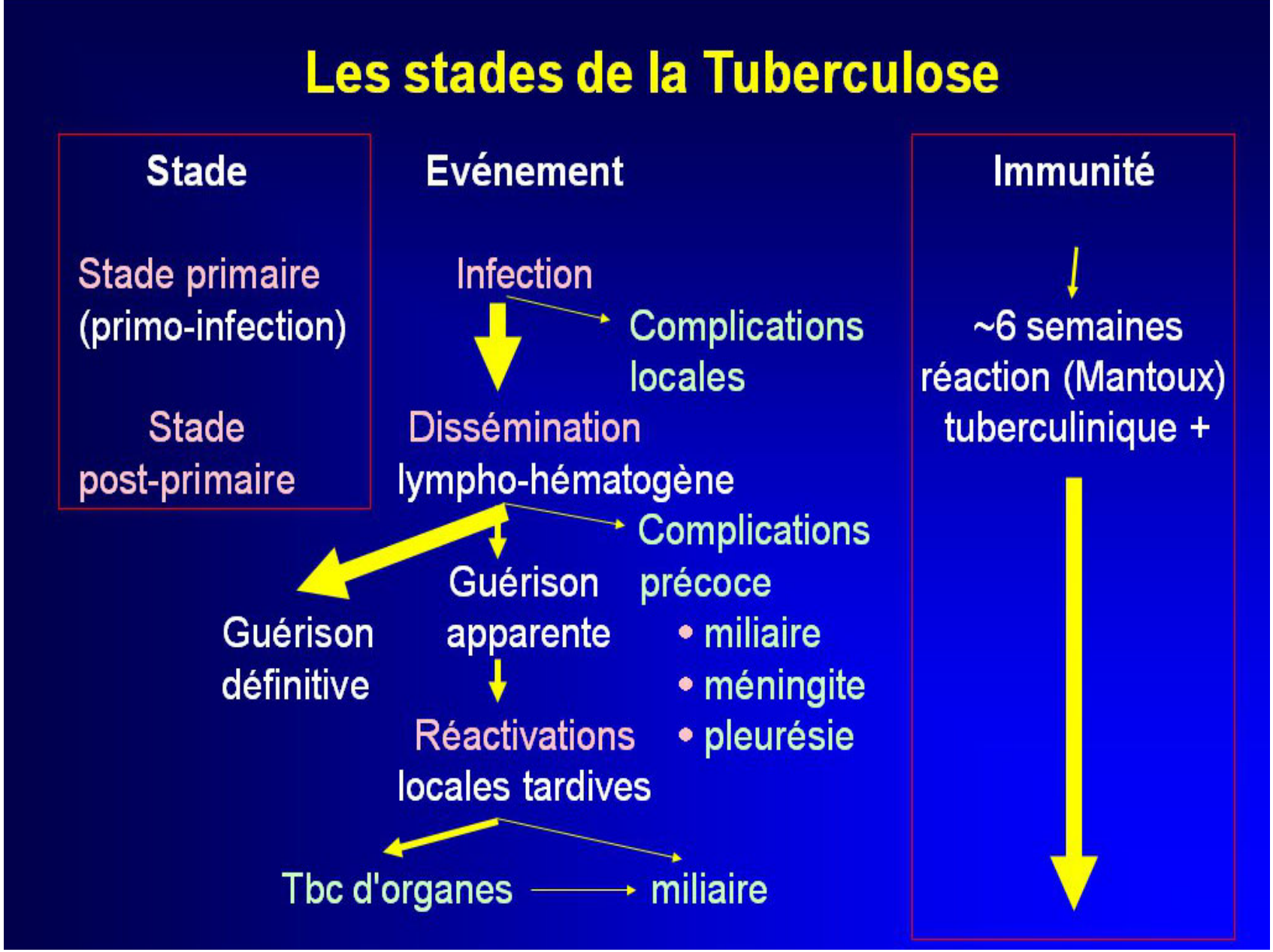
Complication locale du BCG (BCGite)

World Health Organization (WHO)
Philippines 1952
Une grande file de personnes qui attendent de recevoir le vaccin BCG

Physiopathologie



Enveloppe de *Mycobacterium tuberculosis* (10.1038/s41579-019-0273-7)

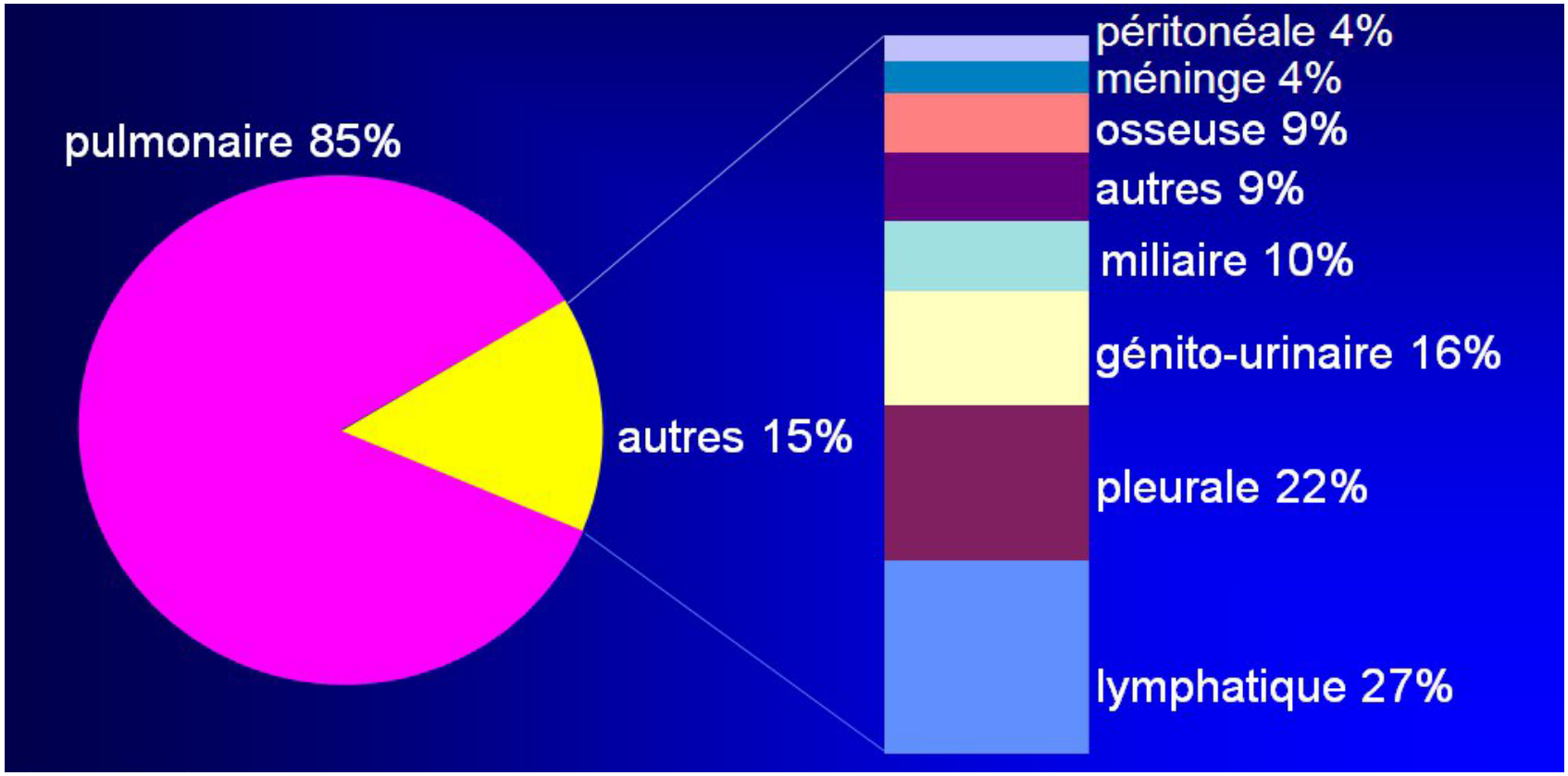

Les stades de la Tuberculose
Stade
Stade primaire (primo-infection)
Stade post-primaire
Evénement
Infection
Complications locales
Dissémination lympho-hématogène
Complications précoce
• miliaire
• méningite
• pleurésie
Guérison définitive
Guérison apparente
Réactivations locales tardives
Tbc d'organes
miliaire
Immunité
~6 semaines réaction (Mantoux) tuberculinique +

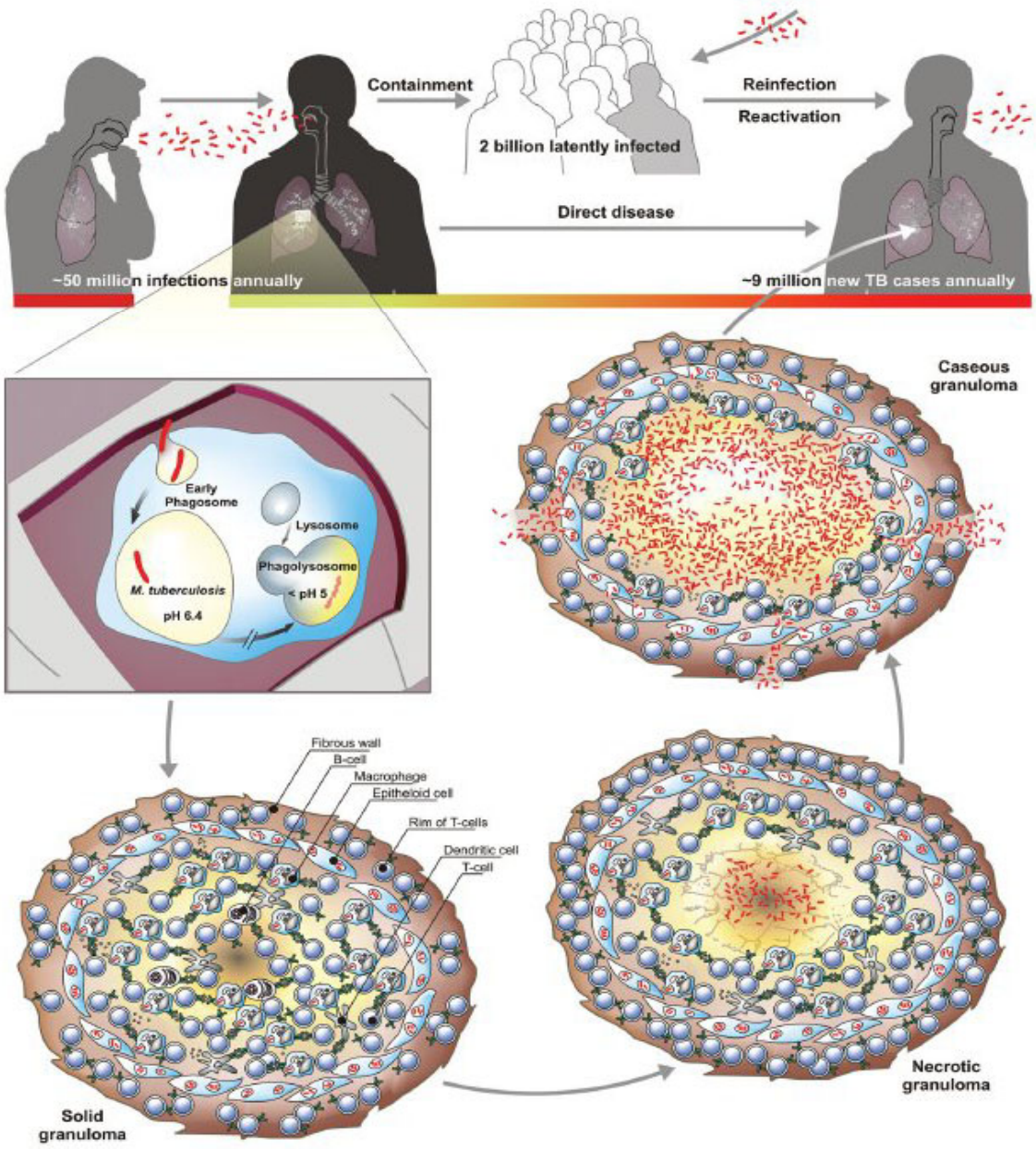
Tuberculose pulmonaire post-primaire:

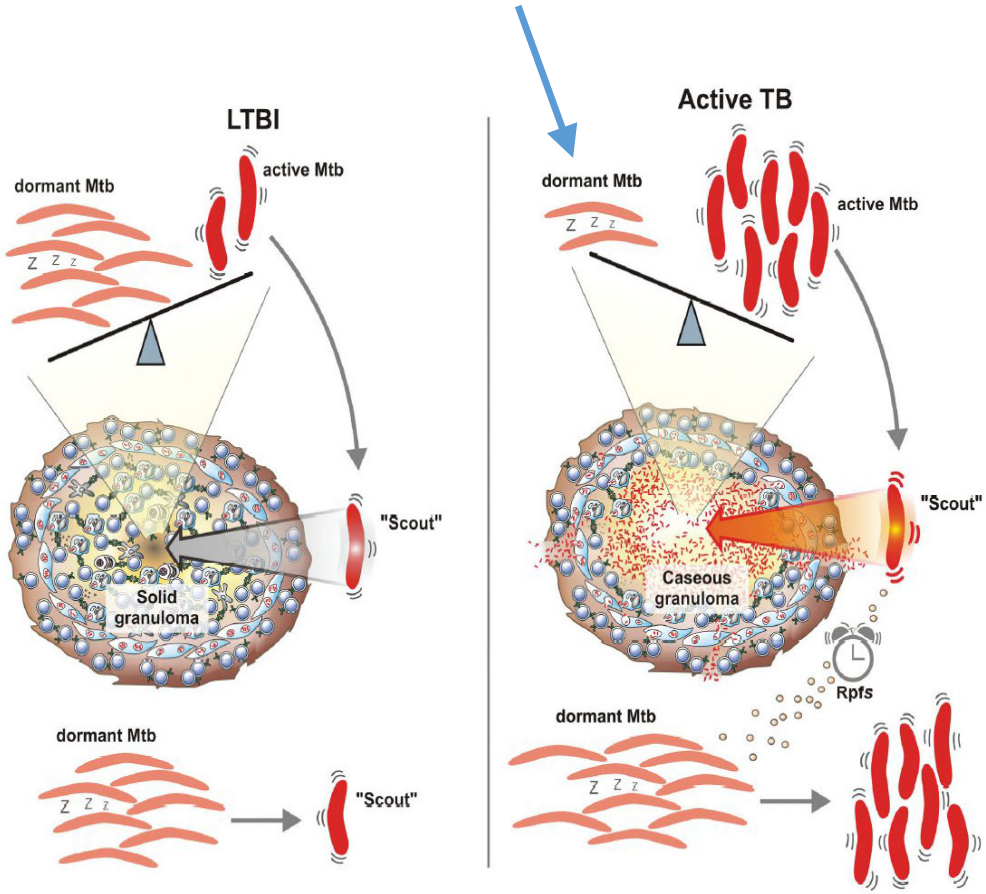
Tuberculose osseuse vertébrale

XX

Distribution des localisations des foyers tuberculeux







(Rpf*s*) Resuscitation promoting factors

doi: 10.1111/j.1574-6976.2012.00331.x

Diagnostic

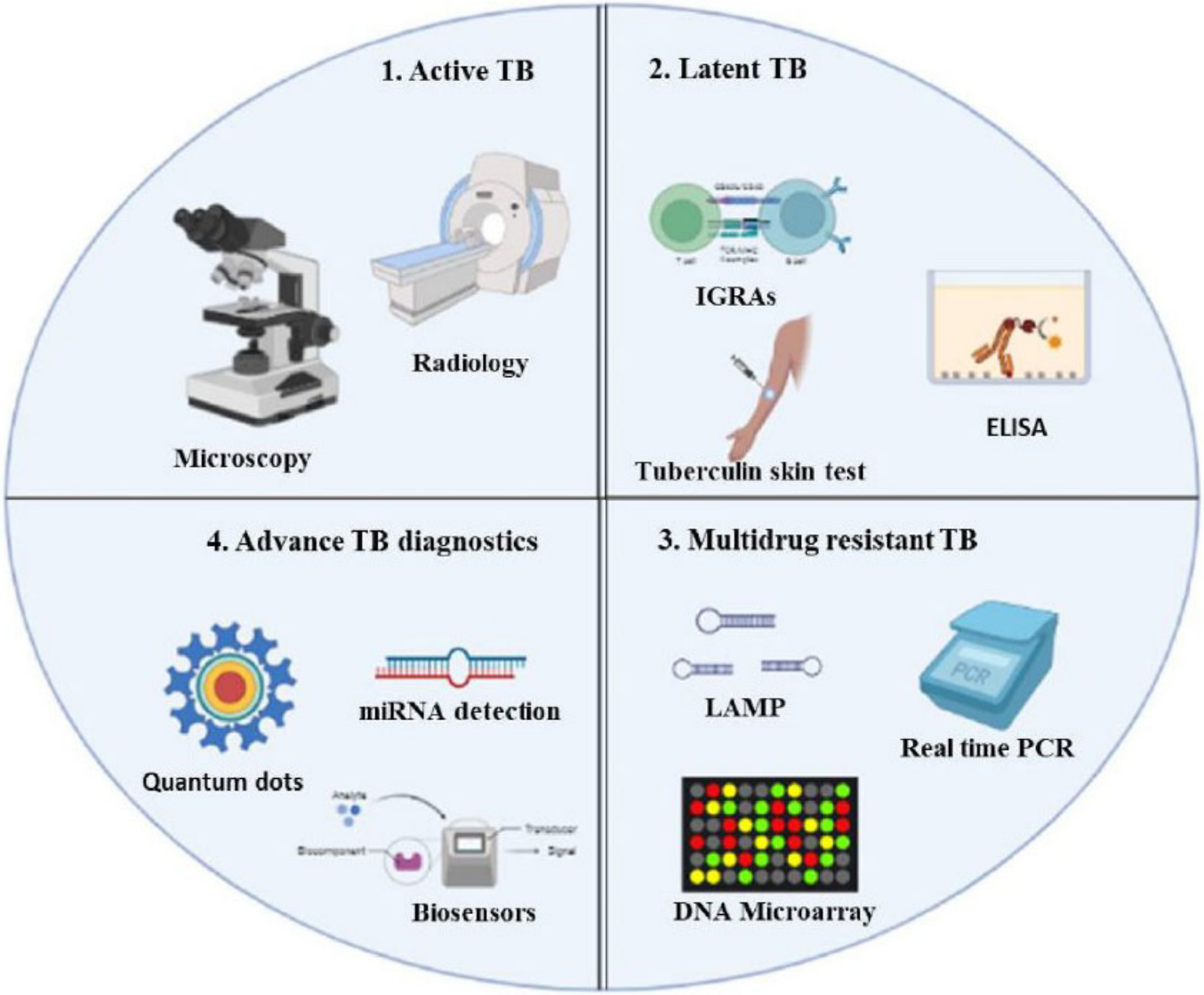
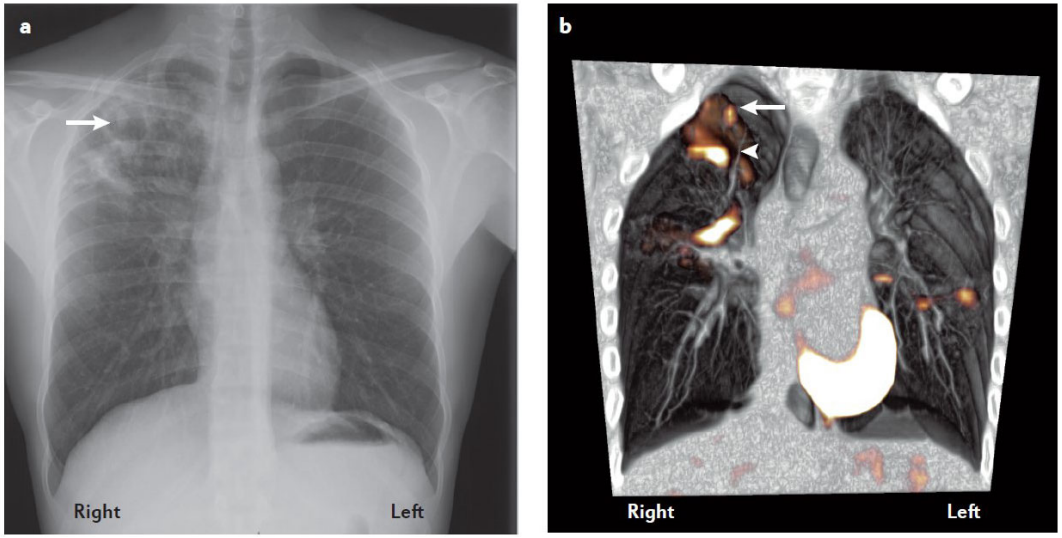


Fig. 3. Diagnostic methods for detection of tuberculosis infection. Early diagnosis of TB is performed using microscopy in which the presence of active bacilli are visualized under microscope and radiology where the chest X-ray is read to detect lesions (1). The Latent TB infection is diagnosed using tuberculin skin test, ELISA and IGRA. The tests are based upon the inflammatory reaction initiated by the host in the presence of *Mtb* pathogen (2). For the diagnosis of multi-drug resistant TB more specific and sensitive assays need to be performed like RT-PCR, DNA microarray and LAMP. These methods specifically determine the mutations in the genes allowing for identification of drug resistance (3). Advancement in diagnosis with the exploitation of nanoparticles, miRNA and CRISPR-Cas brings revolution in TB diagnosis (4).

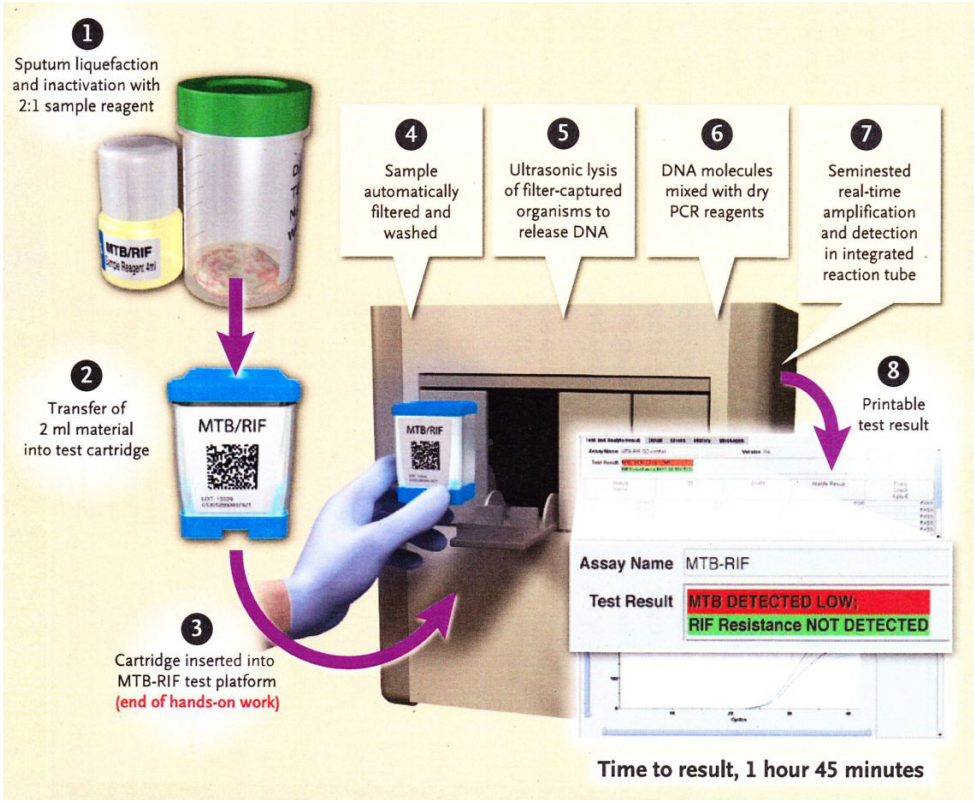
IGRAs: Interferon Gamma Release assays

Méthodes diagnostiques d'une tuberculose active :

- Moléculaires :
  - PCR (Xpert® MTB/RIF Ultra)
  - séquençage génome (antibiogramme génotypique)
- Bactériologiques :
  - examen direct (coloration Auramine ou Ziehl-Neelsen puis observation sous microscope)
  - culture et antibiogramme phénotypique
- Cliniques :
  - Radiographie ou CT-scan thoracique
  - Test d'urine (en cours d'évaluation)



Imaging tools for active TB disease /
doi:10.1038/nrdp.2016.76



Procédure d’analyse du système Xpert MTB/RIF test

Figure 5. Photograph showing the Xpert® MTB/RIF assay being used in a peripheral health facility
Courtesy of National Health Laboratory Service of South Africa.

doi: 10.2217/fmb.11.84

Table 1. Comparison of the results of different diagnostic methods for pulmonary and extrapulmonary samples

Sample type	Auramine O staining		Ziehl Neelsen staining		MGIT culture		Gene Xpert	
	Positive N (%)	Negative N (%)	Positive N (%)	Negative N (%)	Positive N (%)	Negative N (%)	Positive N (%)	Negative N (%)
Pulmonary sample (n=127)	23 (18.1)	104 (81.9)	21 (16.5)	106 (83.5)	26 (20.5)	101 (79.5)	29 (22.8)	98 (77.2)
Extrapulmonary samples (n=48)	7 (14.5)	41 (85.5)	8 (16.6)	40 (83.4)	9 (18.8)	39 (81.2)	11 (22.9)	37 (77.1)
Total (n=175)	30 (17.1)	145 (82.9)	29 (16.6)	146 (83.4)	35 (20)	140 (80)	40 (22.9)	135 (77.1)

Table 2. Comparison of results of staining techniques and GeneXpert with MGIT culture

Tests	Results	MGIT culture		Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Accuracy (%)
		Positive n (%)	Negative n (%)					
Auramine O staining	Positive, n (%)	24 (80)	6 (20)	68.6	95.7	80	92.4	90.3
	Negative, n (%)	11 (7.6)	134 (92.4)					
Ziehl Neelsen staining	Positive, n (%)	23 (79.3)	6 (20.7)	65.7	95.7	79.3	91.8	89.7
	Negative, n (%)	12 (8.2)	134 (91.8)					
GeneXpert	Positive, n (%)	31 (77.5)	9 (22.5)	88.6	93.6	77.5	97.0	92.6
	Negative, n (%)	4 (3.0)	131 (97.0)					

doi: 10.18683/germs.2020.1188

Table 3. Comparison of results of staining techniques with GeneXpert

Tests	Results	Gene Xpert		Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Accuracy (%)
		Positive n (%)	Negative n (%)					
Auramine O staining	Positive, n (%)	28 (93.3)	2 (6.7)	70	98.5	92.2	92.9	92.8
	Negative, n (%)	12 (8.3)	133 (91.7)					
Ziehl Neelsen staining	Positive, n (%)	24 (82.8)	5 (17.2)	60	96.3	80.2	90.6	89
	Negative, n (%)	16 (11)	130 (89.0)					

doi: 10.18683/germs.2020.1188

Table 2
Recent techniques for TB diagnosis.

S. No.	Test	Type	Sensitivity (%)	Specificity (%)	Turnaround duration	Description	References
1.	CRISPR/Cas12a	Gene editing	90%	98%	7 and 14 days	**Advantages** • Detection of *Mtb* in lower sample input • Detect multiple sites in *Mtb* genome associated with drug resistance and susceptibility **Drawbacks** • Cas protein has a lower sensitivity • Target selection is crucial as long target selection near the PAM sequence is advantageous • CRISPR-Cas system is prone to mutation and hence may provide false negative results • Multiplexing is another challenge of CRISPR-Cas	[117,118]
2.	AI Processing	Artificial intelligence	68–96%	72–85%	NA	**Advantages** • Accurate and efficient clinical decision making • Improved precision in screening, diagnosis, and treatment • Reduced labour **Drawbacks** • Need to maintain curative data sets • Privacy concerns, breach of medical data	[119]
3.	MinIONNanopore-sequencing (OxfordNanpore technologies, U.K.)	Detection of Rifampicin resistance supersedes	94.8%	98%	6 h	**Advantages** • Real-time analysis in a scalable format • Minimum sample preparation • Cost effective • Portable • Independent of length of the fragment **Drawbacks** • Resolution should be improved so that it can detect even a single base before commercialization	[120]

4.	Whole-genome sequencing	Next-generation sequencing (NGS)	95%	95%	up to 72 h	**Advantages** • Detect drug resistance and genetic diversity **Drawbacks** • High cost of equipment • Requirement of technical training • Clinical interpretation of NGS data is challenging	[121]
5.	miRNA	PCR based assay	24.7–39.9%	>90%	24 h	**Advantages** • Differential expression of miRNAs represents different states of pathogenesis • Disrupted miRNA expression profile allows in differentiation between active TB and LTBI • Can be performed using diverse sample type such as; whole blood, serum, plasma, PBMCs, pleural fluid, sputum, urine and EBC **Drawbacks** • expression pattern of miRNAs depends on the study design	[122,123, 124]
6.	Raman spectroscopy	Raman scattering	Active TB: 84.62% LTBI:86.84%	Active TB: 89.47% LTBI: 65%	2 h	**Advantages** • Does not require unnecessary sample preparation. • A high degree of intrinsic specificity • Direct measurement can be performed from clinical sample without microbial culture • Easy to use non-invasive • Can detect TB from cerebrospinal fluid samples **Drawbacks** • Adoption of Raman spectroscopy in a clinical lab is a challenge	[125]
7.	Aeonose (eNose BV, Netherlands	Detection of VOC (breath analyzer)	75–92%	44–65%	10 min	**Advantages** • High specificity • Shorter test time • Immediate data analysis	[126]

Zein-Eddine *et al.*
Journal of Biomedical Science (2023) 30:34
https://doi.org/10.1186/s12929-023-00932-4

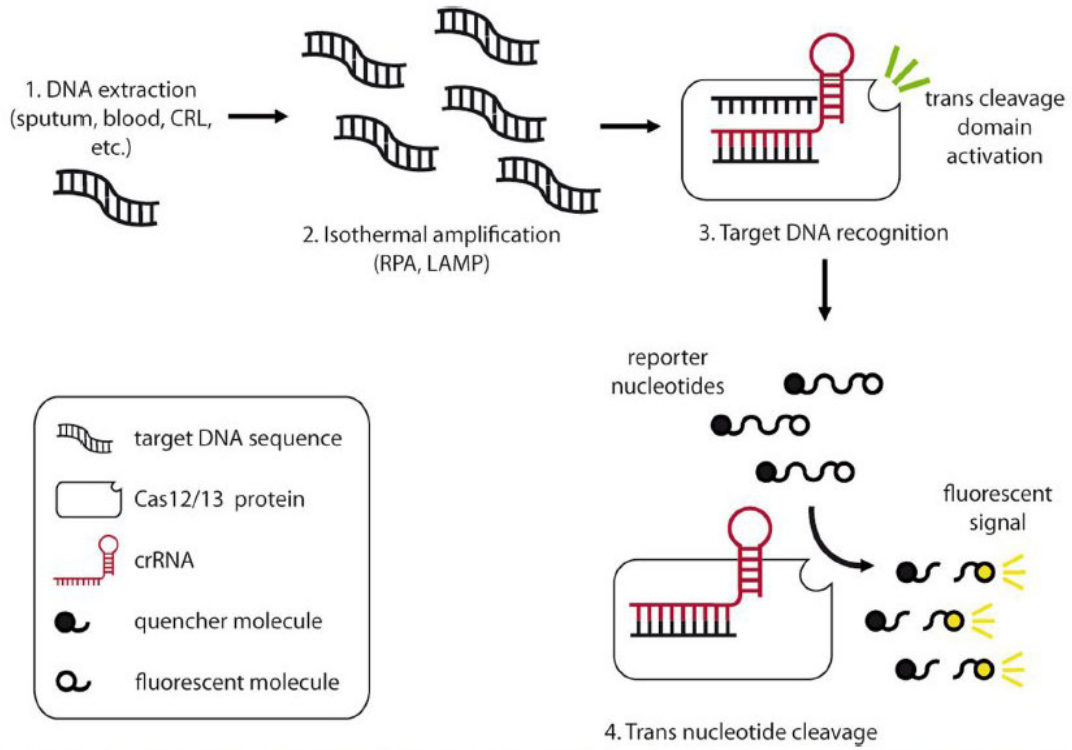
Journal of Biomedical Science

REVIEW Open Access

The future of CRISPR in *Mycobacterium tuberculosis* infection

Rima Zein-Eddine[1], Guislaine Refrégier[2], Jorge Cervantes[3] and Noemí Kaoru Yokobori[4,5*]

Clustered Regularly Interspaced Short Palindromic repeats (CRISPR)-Cas systems



Sensitivity (%)	Specificity (%)	Turnaround duration
90%	98%	7 and 14 days

Advantages

- Detection of *Mtb* in lower sample input
- Detect multiple sites in *Mtb* genome associated with drug resistance and susceptibility

Drawbacks

- Cas protein has a lower sensitivity
- Target selection is crucial as long target selection near the PAM sequence is advantageous
- CRISPR-Cas system is prone to mutation and hence may provide false negative results
- Multiplexing is another challenge of CRISPR-Cas

Box 1. Challenges for the massive application of CRISPR in tuberculosis diagnosis

1) DNA extraction methods.
2) Automation and closed system to prevent crossed contamination.
3) Development of POC tests such as lateral immunochromatography.
4) Affordability.
5) Automated crRNA design tools for SNP-level discrimination.
6) Multiplexing.

Infections cutanées à Mycobactéries atypiques

Yoo et al. BMC Infectious Diseases 2013, 13:479
http://www.biomedcentral.com/1471-2334/13/479

BMC Infectious Diseases

CASE REPORT Open Access

Facial skin and soft tissue infection caused by *Mycobacterium wolinskyi* associated with cosmetic procedures

Seung Jin Yoo[1†], Keun Hwa Lee[2†], Sung-No Jung[3†] and Sang Taek Heo[4*]

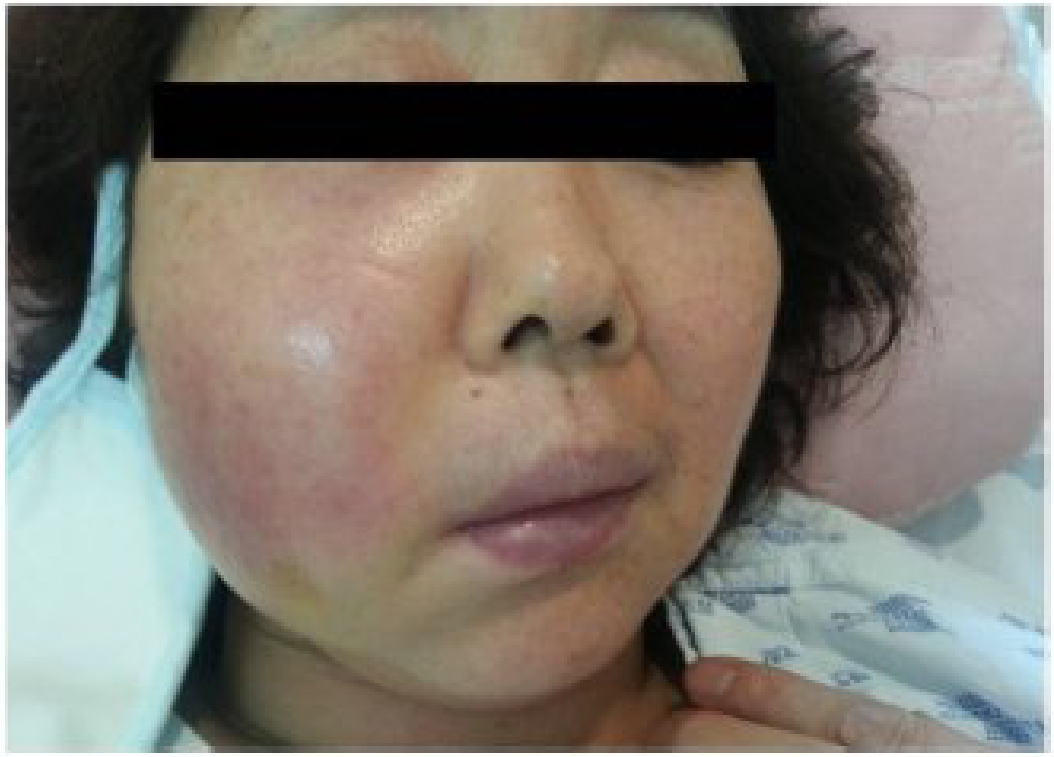


Figure 1 Small indurated nodules with persisting edema on the right cheek.

Merci pour votre attention