TECHNICAL EVALUATION OF TWO RAPID KITS FOR RABIES DIAGNOSIS

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Objective

Assessment of the technical performance of two commercially available rapid immunochromatographic tests for rabies diagnosis, and to compare it to the Fluorescent Antibody Test

Characteristics :

- immunochromatographic tests = Lateral Flow Assays (LFAs) = Lateral Flow Device (LFDs)
- generally based on colloidal gold conjugated monoclonal antibodies that capture the antigen contained in a sample
- The antigen–antibody complex migrates on a nitrocellulose membrane and binds to a detection antibody fixed in the test zone "T" revealing a coloured line for a positive sample.
- Simple, rapid (results obtained in about 5 to 10 minutes) and that they do not request any special equipment

Samples

Sample status	Country of origin	Infection origin	Number of samples
Rabies positive	Morocco	natural	20
	Ukraine	natural	9
	Hungary	natural	4
	Serbia	natural	6
	Laboratory*	experimental	7
Rabies Negative	France	-	45

** Brains collected from mice experimentally infected with RABV or other lyssaviruses, and subsequently lyophilized*

91 brain samples were tested (46 rabies + and 45 rabies -)

LFD kits

- Petscreen *Canine Rabies antigen test®*, from Global DX ltd, United Kingdom,
- Redtest *rabies virus antigen rapid test®*, Sigmed, Poland.

Intented use:

Petscreen = saliva and brain homogenates

Redtest = saliva and cerebrospinal fluid (=> brain specimens)

Results : FAT vs Global DX LFD

		FAT results	
		Positive	Negative
LFD results	Positive	3	0
	Negative	43	45

Specificity of 100% (95% CI: 92.1 - 100%)

Sensitivity of only 6.5% (95% CI: 1.37 - 17.9%)

Only 3 FAT positive samples (2 from Morocco and on freeze-dried sample infected with ABLV) were confirmed positive with this LFD

Results : FAT vs REDTEST LFD

		FAT results	
		Positive	Negative
LFD results	Positive	37	0
	Negative	9	45

Specificity of 100% (95% CI: 92.1 - 100%)

Sensitivity of only 80,4% (95% CI: 66.1 - 90.6%)

Redtest did not succeed to detect rabies antigens from the samples experimentally infected with Duvenhage, EBLV-1a, EBLV-1b, EBLV-2 and BBLV

Results

Agreement between FAT and LFD results (qualitative results)

For each test, comparison between FAT and LFD (Kappa statistic test) =>Determination of a Cohen's Kappa values

- FAT *vs* Global DX LFD results = poor agreement (Cohen's kappa = 0.065 - CI: 0 – 0.136) between the two tests.
- FAT *vs* Redtest LFD results = a good agreement (Cohen's kappa = 0.802 - CI: 0.682 – 0.923) between the two tests.

Kappa score	Interpretation
< 0	No agreement
0.0 — 0.20	Slight agreement
0.21 — 0.40	Fair agreement
0.41 — 0.60	Moderate agreement
0.61 — 0.80	Substantial agreement
0.81 — 1.00	Almost perfect agreement

LFD evaluations for rabies diagnosis

> 20 publications/reports from 2007 – 2021

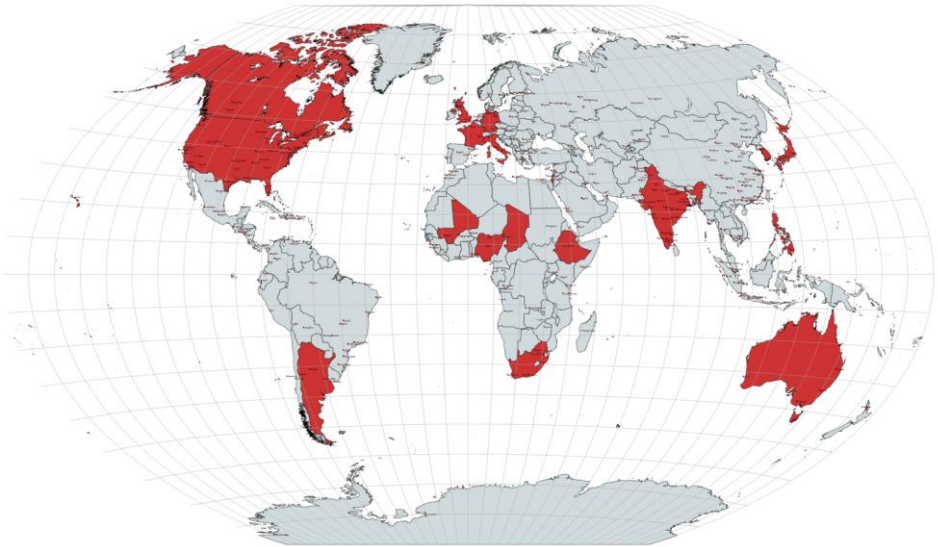
> 1870 brain samples analyzed (LFD vs DFA), collections from about 20 countries

13 different LFD kits evaluated *(Anigen test from Bionote part of 17 studies)*

Performance :

Specificity generally equal to 100% (except 4 studies with sp between 93% and 99%).

Sensitivity quite heterogeneous according to kits



LFD for rabies diagnosis evaluations

8 kits with sensitivities ranging from 0 to 20%

Ubio - 0% (Eggerbauer et al. 2016)

Quicking - 0 to 6,6% (Eggerbauer et al. 2016)

Biogen - 0 to 2,2% (Eggerbauer et al. 2016)

Elabscience - 0 to 20% (Kimitsutmi et al. 2020, Klein et al. 2020)

Intermedical - 3% (Klein et al. 2020)

Lillitest - 1% (Klein et al. 2020)

Span Biotec - 0% (Klein et al. 2020)

Global DX - 6,5% (Servat & Robardet, unpub, 2020)

2 kits with sensitivities ranging from 22 to 70%

Creative Diagnostic – 22,7 to 70% (Eggerbauer et al. 2016)

GreenSpring – 31,8 to 50% (Eggerbauer et al. 2016)

13 different LFD evaluated

3 kits with sensitivities ranging from 80 to 100%

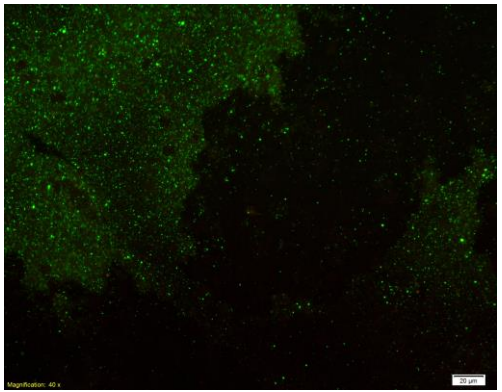
ADTEC – 88 to 94,3% (Kimitsutmi et al. 2020, Manangitt et al. 2021)

Redtest – 80,4% (Servat & Robardet, unpub, 2020)

Anigen – 88,3 to 100% (except in 2 studies : 62% & 16% to 100%)

Conclusion

- ✓ Petscreen®: barely unable to detect rabies antigens in positive samples (93.5% of false negative) + poor agreement with FAT
- ✓ Redtest® : good performance (sp, se, agreement with FAT) on RABV infected brain specimens of various animal species, but failed to detect most of all other non-RABV lyssaviruses.
- ✓ Confirmation of the great heterogeneity of LFD performance.
  - ➢ Need for improvement of rapid tests
  - ➢ Need for batch to batch consistency
  - ➢ Need for more transparency of reagents used by manufacturers (Mabs etc.)
  - ➢ Need for qualification of kits before being used/authorized



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Thank you for your attention !