

## Protocol for the detection of Monkeypox by RT-PCR

This RT-PCR allows the detection of members of the genus *Orthopoxvirus* including *Monkeypox*, *Cowpox* and *Vaccinia virus*.

### 1. Extraction

External lysis (BLS-3): 400 ul of samples and 1 ml of lysis buffer (EMag, Biomérieux)  
Internal control: 20 ul of CDV (baculovirus containing a fragment of the fusion gene of *Canine distemper virus*)  
EMag extraction according to manufacturer's instruction with elution volume of 50 ul

### 2. RT-PCR

Target	Primers/probes	Sequences (5'-3')	Final conc. (nM)
Orthopox	GF (for)	GCCAGAGATATCATAGCCGCTC	600
	GR (rev)	CAACGACTAACTAATTTGGAAAAAAGAT	900
	14kdPOX (probe)	FAM-TTTTCCAACCTAAATAGAACTTCATCGTTGCGTT-TAMRA	250
CDV	CDV 1 (for)	GCTACCCAAGAAACCGTCATTG	900
	CDV 1 (rev)	GCATGGCAGGGACGAGTT	900
	CDV (probe)	VIC-CGTTCAGGGAGTCCAGGACTACGTCAAC-TAMRA	200

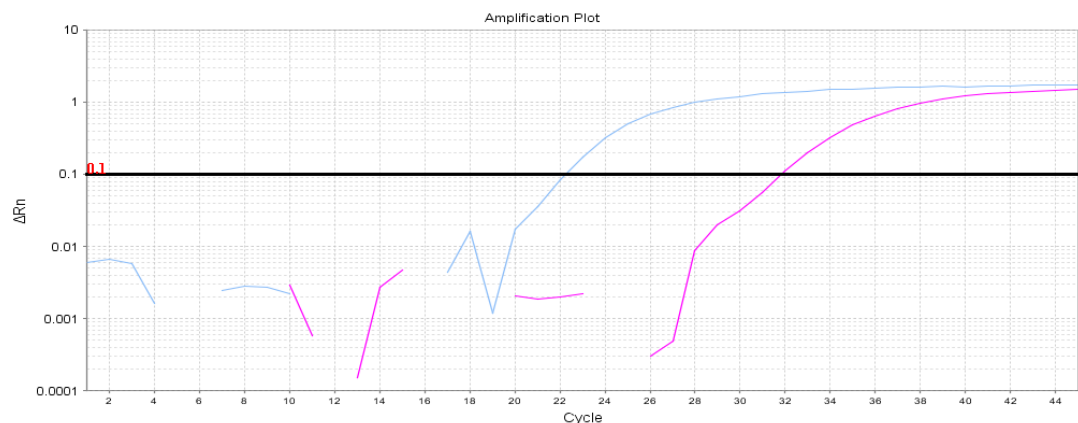
Positive control: plasmid pVACV-List143

Orthopox      10 ul TaqMan mix 2x (Applied Biosystems, ref : 4304437)  
                  5 ul primers/probes Orthopox  
                  5 ul extraction or control

CDV            10 ul TaqMan mix 2x (Applied Biosystems, ref : 4304437)  
                  5 ul primers/probe CDV  
                  5 ul extraction

Cycles:        50C 2 min  
                  95C 10 min  
                  95C 15 sec, 60C 1 min x 45

### 3. Results (StepOne with threshold : 0.1)



### 4. Reference : Scaramozzino-N et al. Clinical Chemistry 2007; 53 :606-613