

Validation of an in-house developed Real-Time Multiplex for Respiratory Viruses

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Motivation and test method/ assay information: Introduction

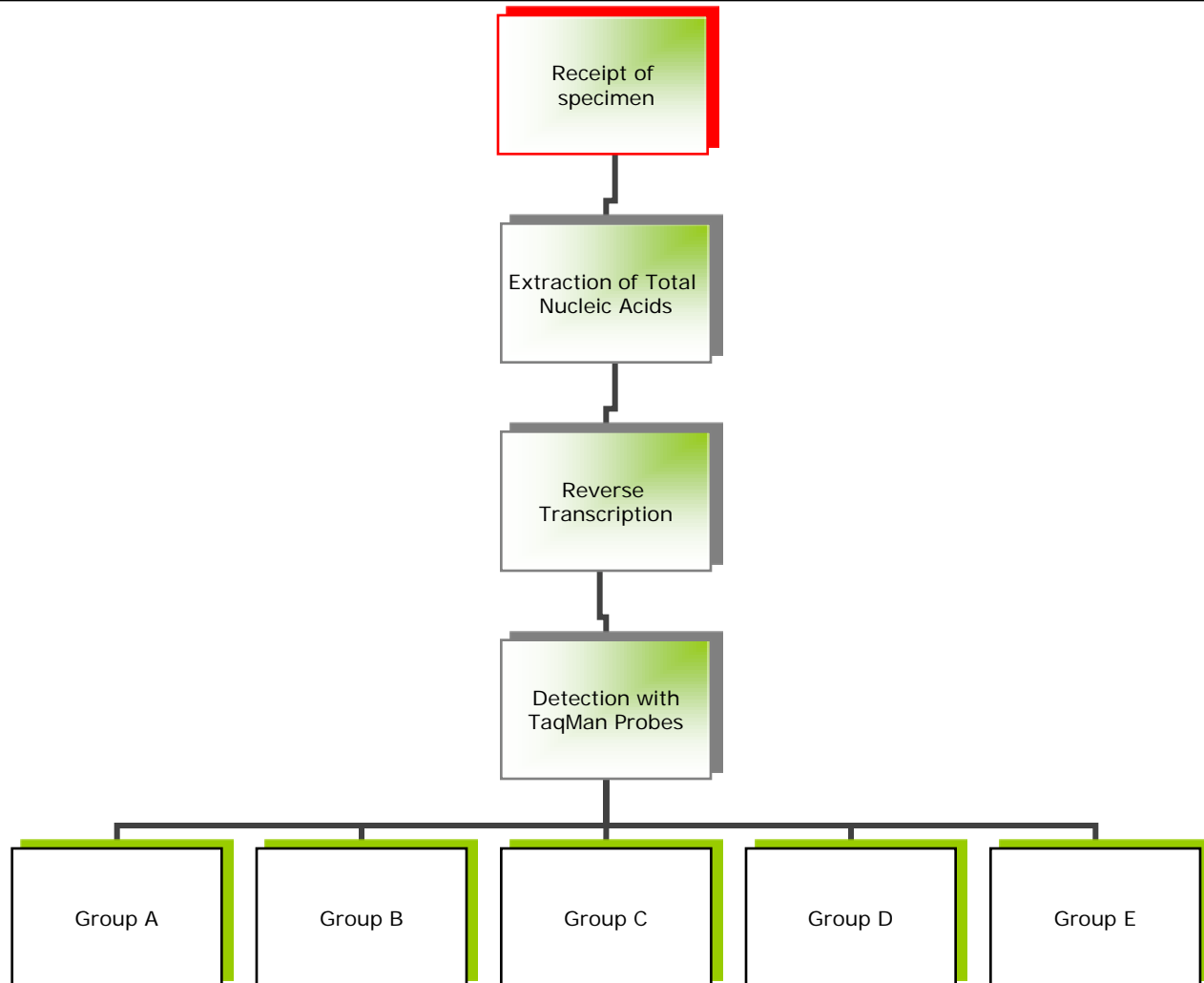
- Severe Acute Respiratory Infection (SARI) is estimated to cause approximately 20% of all under 5 deaths in South Africa, and 30-40% of hospital admissions in this age group.
- The rate of hospitalisations due to pneumonia has increased in all age groups due to increasing prevalence of HIV infection.
- Influenza is estimated to result in up to five million cases of severe illness and 250,000 to 500,000 deaths worldwide each year.



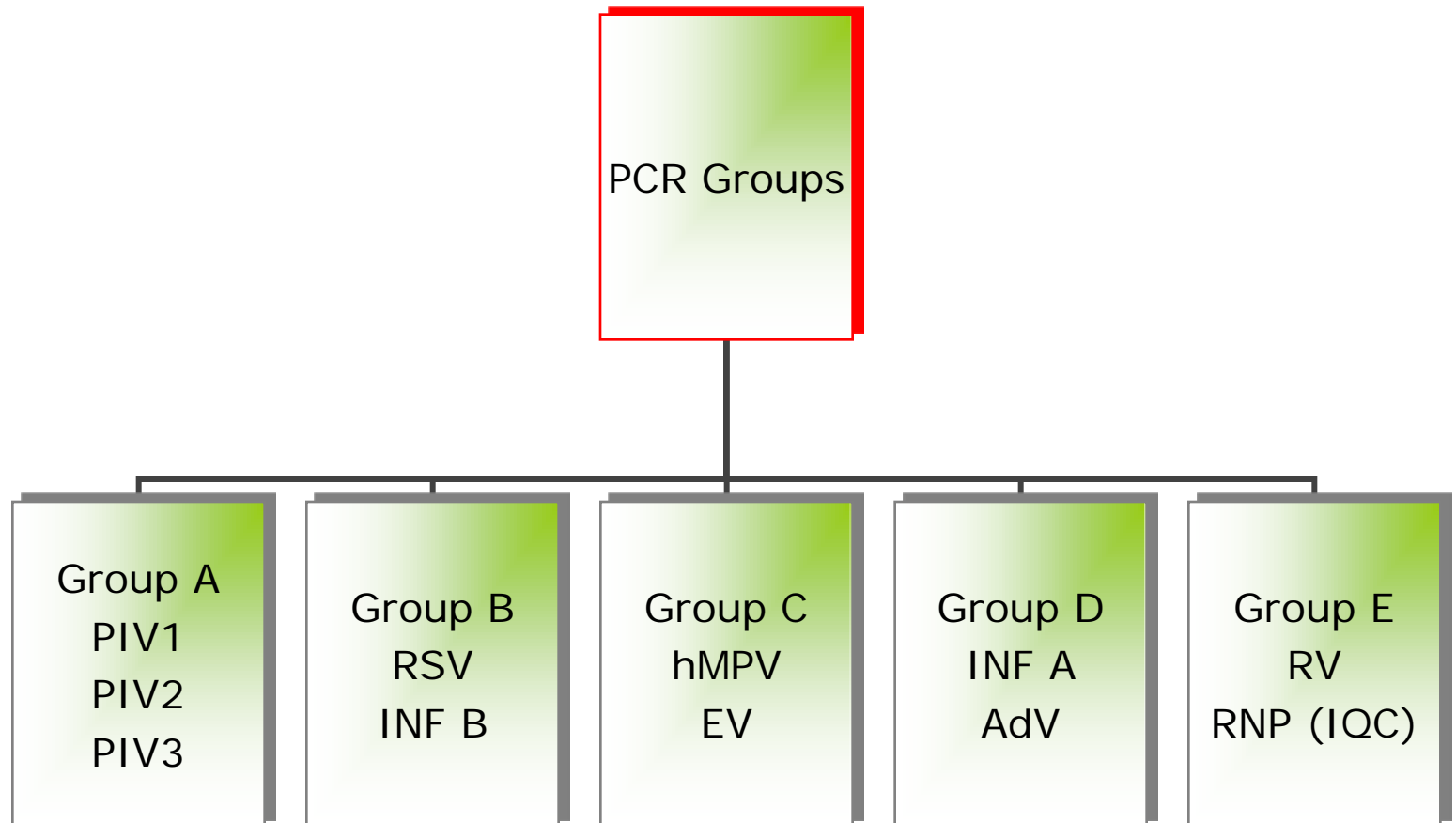
Introduction

- There is limited data regarding the burden and the impact of influenza and the other respiratory viruses in Africa.
- The SARI surveillance study aims to:
 - determine the contribution of influenza and other respiratory viruses to the burden of disease,
 - determine seasonality of the other respiratory viruses in South Africa.

Procedure



PCR Groups





Criteria and Expected Results

- Final QCMD report for 2002:
 - within the expected range of 2SD,
 - correlate well with the other laboratories using same technology.
- Overall Accuracy (97.8%), Negative Predictive Value (97.2%), Positive Predictive Value (100%), Sensitivity (91%) and Specificity (100%).

Results: using EQA panels as specimens with known results

PCR Group	Accuracy	NPV	Sensitivity	PPV	Specificity
A	98.3%	97.7%	93%	100%	100%
B	98.3%	98%	91.3%	100%	100%
C	97.2%	97%	87%	100%	100%
D	96%	94.3%	89%	100%	100%
E	99%	99%	94%	100%	100%

Technology Comparisons

Kappa values for Culture	
RSV	0.93
AV	0.24
PIV	0.62
INF	0.83

A subset of specimens were cultured and the results were compared to that of the assay.

These results correlate well, with published literature.

Bland-Altman plots for Multiplex and Seeplex				
Virus	Bias	Limits of agreement		SD
AV	0.135	-0.073	0.343	0.016
EV	0.091	-0.02	0.203	0.057
PIV	0.062	-0.046	0.17	0.055
RSV	0.047	-0.035	0.13	0.042
RV	0.039	-0.01	0.088	0.025
INF	0.053	0.033	0.074	0.011

Detection Limit

Virus	Ct value	2 SD	Percentage Detection
PIV 1	29	0.34	100
	32	0	10
PIV 2	32	0.27	100
	33	0.57	100
	35	0	10
PIV 3	32	0.8	100
	34	1.15	100
RSV	31	0.58	100
	35	1.49	80
	36	1.02	40
INF B	33	0.96	100
	35	0.55	40
hMPV	27	2.42	100
	29	2.7	40

Virus	Ct value	2 SD	Percentage Detection
EV	27	1.88	100
	28	2.51	80
	29	1.25	40
INF A	33	1.11	100
	34	0.1	40
AV	32	0.99	100
	33	0.17	40
RV	28	0.31	100
	29	1.05	60



Cross reactivity

- There are no cross reactivity between the PCR Groups,
 - With the exception of Group E (Rhinovirus) and Enterovirus.
- RV and EV are closely related,
 - To accommodate all the subtypes make use of conserved areas - RV cross reacts with EV.
- EV PCR which does not cross react with RV is used to distinguish the EV positive from RV positives.



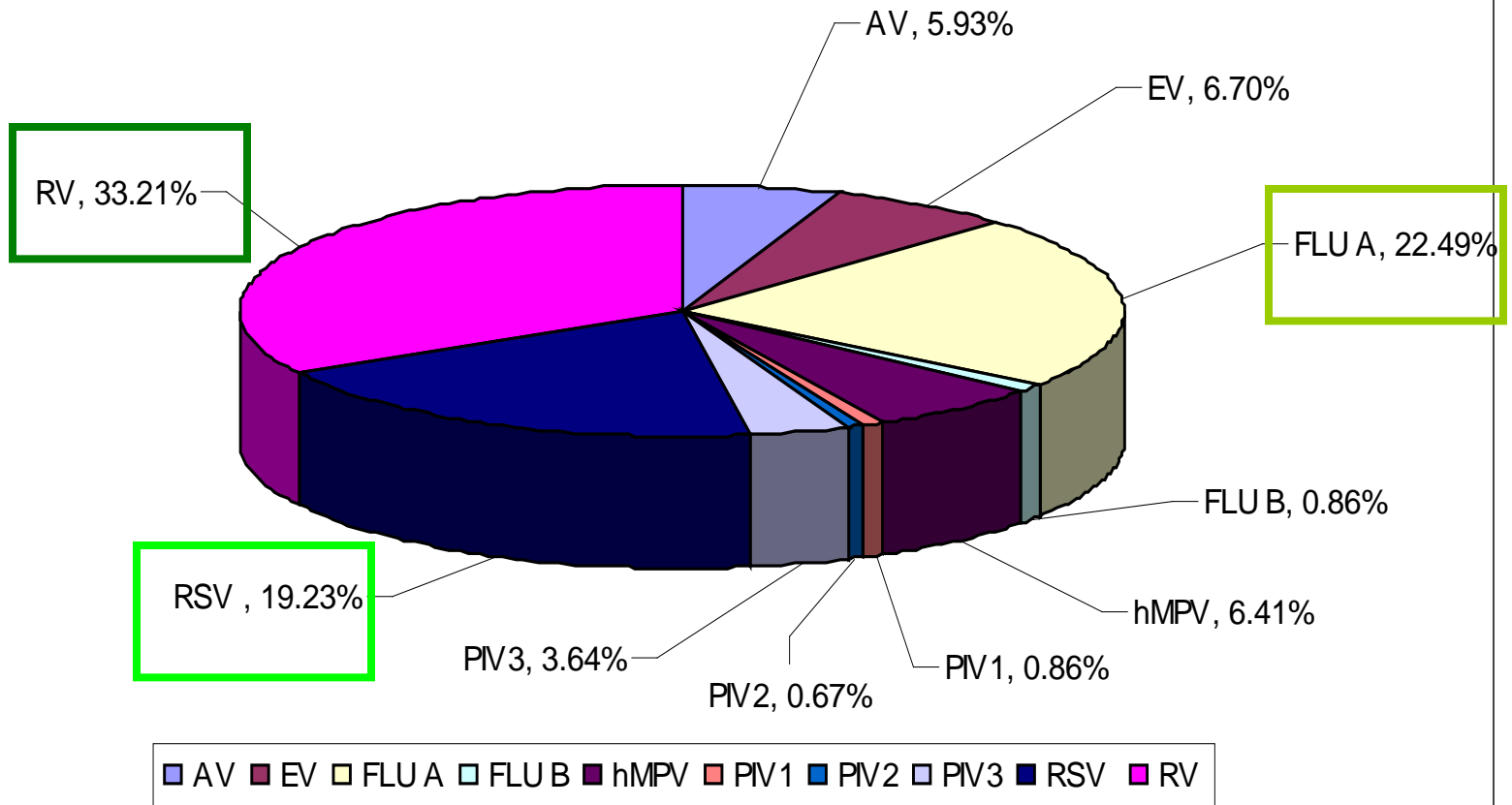
Uncertainty of measurement

- PCR efficiencies
- Validated for VTM containing nasal aspirates, nasopharyngeal, throat swabs and cell cultured virus.
- Collection, transport of specimen, storage and processing procedures.
- Amplicon contamination
- Competent personnel.

Preliminary Data: SARI Single Infection Distribution

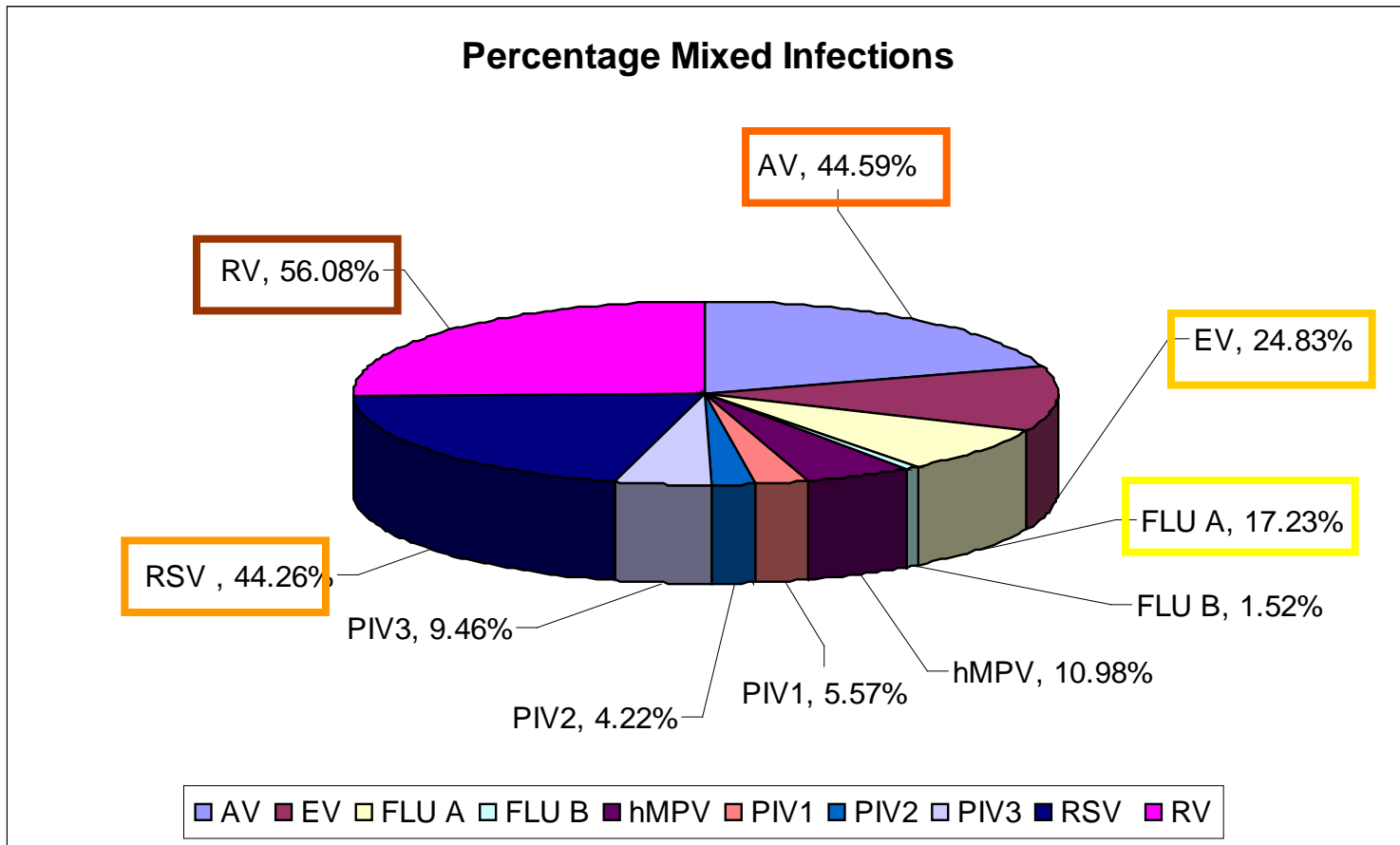
n = 1045

Percentage Single Infections



Mixed Infection Distribution

n=592





Conclusion

- Validation of molecular assays is complicated and there is continuous evaluation of the method with EQA but to date comparable data has been observed when comparing to cell culture methods and other established PCR methods with exceptions.
- The PPV and specificity at a 100 % indicates that the assay is adequate in that it does not give any false positive results.
- The NPV (97.2%) and sensitivity (91%) is determined by the lower detection limit of this assay, this assay gives false negatives with specimens with low viral load
- The sensitivity and specificity together with NPV and PPV indicates that this assay is more than suitable for the detection of Respiratory Viruses in clinical specimens.



Conclusion

- Preliminary data suggests that the 3 viruses were detected most frequently: RSV, RV and Influenza A.
- That the distribution for the other respiratory viruses does match those of the Northern Hemisphere
- The incidence of Rhinovirus is increased as it is present in most of the mixed infections, especially with RSV.
 - Is Co-infection meaningful in severe disease?
- The assay design does not yet allow for distinguishing which disease causing agent is dominant/causative but will contribute to understanding respiratory viruses' patterns across the seasons that contribute to SARI.



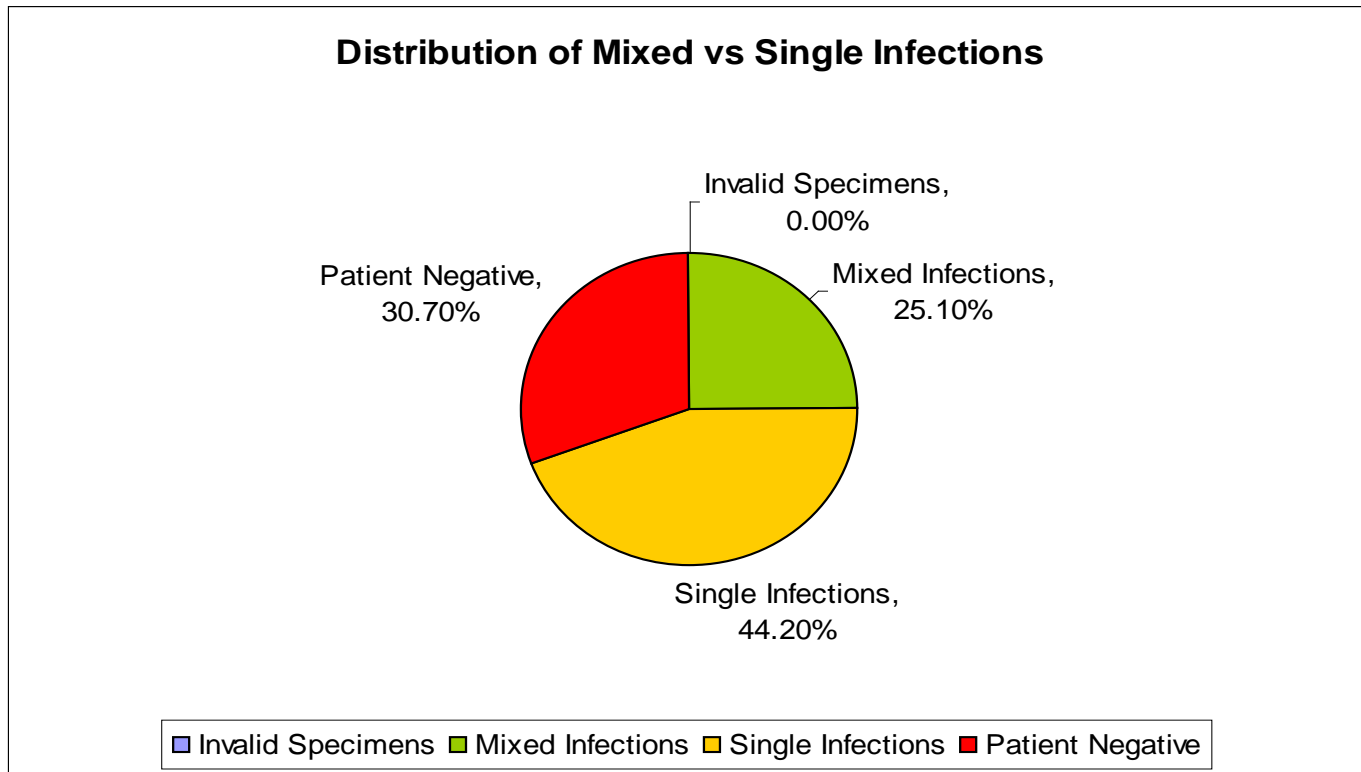
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Preliminary Data for SARI

- Distribution for Mixed and Single Infections

n = 2634



AdV Bland-Altman plots

